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# Electron microscopic analysis in the gecko *Lygodactylus* reveals variations in micro-ornamentation and sensory organs distribution in the epidermis that indicate regional functions

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**Abstract**

Possible pattern variations of micro-ornamentation in different areas of the skin in the gecko *Lygodactylus* have been analyzed by scanning and transmission electron microscopy. A map of micro-ornamentation present in various areas of the skin has been obtained. Differences in micro-ornamentation pattern and sensory organ distribution were detected. The "spinulated pattern" consists of shorter spinulae in dorsal versus ventral scales, and spinules are shorter in inner scale surface and hinge regions with respect to the outer scale surface. The spines derive from the accumulation of struts of corneous material mainly composed of corneous beta proteins (CBPs, formerly indicated as beta-keratins) that merge into pointed micro-ornamentation. The 3D-accumulation of CBPs within Oberhautchen cells can vary in some regions of different scales during Oberhautchen-beta cell differentiation, perhaps also under physical tensile forces derived from continuous scale growth. Three other main patterns of micro-ornamentation were detected and indicated as "corneous belts," "corneous dendritic ramification," and "serpentine-pit and groove." These variations from the typical spinulated pattern present in gecko epidermis are interpreted as transitional regions where the accumulation of corneous material in Oberhautchen cells that merges with underlying beta-cells gives rise to nonspinulated surfaces. Spinulated sensory organs with bristles and lenticular-shaped or knob-like tactile corpuscles are more numerous in ventral scales of the tail tip close to adhesive pads and near the digital pads. These regions are likely those most involved in the fine control of movements and response to vibrational stimuli derived from air and objects movements, including potential preys or predators.

**KEYWORDS**

gecko, map, micro ornamentation, SEM, skin

## 1 INTRODUCTION

The more superficial part of the epidermis in lizard and snake scales forms numerous species-specific patterns of micro-ornamentation (microstructures or micro-dermatoglyphics) that derive from the variable process of accumulation of corneous material in the Oberhautchen layer of the epidermis (Alibardi, 1999, 2002; Irish et al., 1988; Maderson, 1970; Maderson et al., 1998; Price, 1982; Ruibal, 1968). During the formation of the epidermis, cells of the Oberhautchen layer accumulate mainly corneous beta proteins (CBPs, formerly indicated as beta-keratins) on their external surface that is joined by desmosomes to an external layer of cells devoid of these proteins, indicated as clear layer (Alibardi, 2002, 2003, 2016; Landmann, 1979; Maderson et al., 1972, 1998). The cytoplasm of the clear layer somehow molds the surface of Oberhautchen cells generating protrusions of different shape and length that give rise to micro-ornamentation. A complex cytoskeletal networks that include tubulin and actin is likely involved in the formation of micro-ornamentation, in particular for the long bristles indicated as setae that are typical of the adhesive digital or tail pads of geckos (Alibardi, 1999, 2020; Alibardi & Bonfitto, 2019; Bauer, 1998; Griffing et al., 2021; Hiller, 1972; Maderson, 1966, 1970). The Oberhautchen layer matures forming a compact corneous layer that is involved in the separation from the clear layer in the process of shedding with the release of a molt.

It has been proposed that initially the surface of lepidosaurian epidermis was relatively smooth and unpatterned but that it evolved different patterns in diverse families of lizards and snakes in relation to the adaptation to various environments. It is also likely that micro-ornamentation of different types derived from the specific process of corneous material accumulation that took place during the evolution of the shedding complex in different lepidosaurians. The role of the corneous microstructures formed by the Oberhautchen varies according to the species but it has been indicated to function in different processes such as optimize the timing of epidermal shedding, producing skin friction of anti-friction, generating shining and iridescence, increasing infra-red wavelengths absorption for thermoregulation, removing fouling, dirt particles or microorganisms and parasites from the skin (Arnold, 2002; Gower, 2003; Riedel et al., 2019; Ruibal, 1968). The variable patterns of Oberhautchen surfaces so far analyzed in lizards and snakes by Scanning Electron Microscopy (SEM), have been briefly summarized to form five main types: flat-smooth, lamellated, lamellated-spinulated, spinulated, and honeycomb (Irish et al., 1988; Peterson, 1984; Price, 1982; Ruibal, 1968). However, extreme variations of micro-ornamentation patterns have been observed in some geckos that do not complain with the above five main types (Gasc et al., 1982; Gasc & Renous, 1980).

In addition to micro-ornamentation, also variations and possible roles of sensory-tactile organs in different skin areas of numerous lizards and some snakes have been analyzed (Ananjeva et al., 1991; Dujsebayeva, 1995; Dujsebayeva et al., 2021; Harvey & Gutberlet, 1995; Hiller, 1977; Jackson, 1977; Lang, 1989; Matveyeva & Ananjeva, 1995; Povel & Van Der Kooij, 1997; Riedel & Schwarzkopf, 2022; Russell et al., 2021; Sherbrooke & Nagle, 1996; Von During & Miller, 1979). It is however incompletely known whether different microstructures over the entire skin in lizards and snakes may reflect differences in local functions, in particular in association with sensory organs, since most studies only deal with small epidermal areas of the body.

A family of lizards, the gekkonids, shows a general pattern of micro-ornamentation indicated as "spinulated" since it is composed of an external surface that is variably covered with short spiny protrusions of 0.5–3 μm in length (Griffing et al., 2021; Ruibal, 1968; Spinner et al., 2013; Stewart & Daniel, 1973). Numerous geckoes possess an extension of this pattern, increasing the size of spines in the digital pads and in some caudal scales, and forming long bristles or setae that allow adhesion of these agile liz-ards to different substrates, defying gravity (Alibardi, 2018; Alibardi & Bonfitto, 2019; Autumn & Peattie, 2002; Bauer, 1998; Griffing et al., 2021; Irish et al., 1988; Maderson, 1970; Niewiarowski et al., 2016; Russell, 2002). These studies have however indicated that the spinulated micro-ornamentation pattern is present in few representative scales of geckos, dorsal and ventral mainly, but an analysis of possible variations of the pattern in different body regions, forming a map of their superficial distribution on the body of a single lizard, is still missing.

As in the digital and tail pads the formation of long setae is correlated to climbing ability, it is possible that other types of micro-ornamentation variations have other functions, in addition to the distribution of skin sensory organs. To address this problem, the present SEM study aims to reveal a possible variation in the micro-ornamentation pattern and distribution of sensory organs in various body regions of a small African gecko, *Lygodactylus conraui*. This prevalently diurnal species lives in Central-Western Africa (Cameroon, Sierra Leone, Equatorial Guinea, Ghana, Gabon, Benin, South Nigeria), and is an arboreal species than can adapt from savannahs and open forests to humid and swampy forests and also in areas of reforestation (Amadi et al., 2017). This species can also adapt to anthropic urban settings (Manners & Georgen, 2015). The study also includes another small African gecko, *Lygodactylus capensis*, whereas only the tail pads were analyzed for comparison with the digital pads of the former species (Alibardi & Bonfitto, 2019). This genus of small geckos not only develops tail pads during embryo-genesis and epidermal renewal but can also regenerate them after tail amputation (Alibardi & Bonfitto, 2019; Maderson, 1971). The present survey aims to obtain a gen-eral skin map of the micro-ornamentation present in this species in order to evaluate whether different skin regions have specific roles during the movements of this gecko across forest canopy or more open spaces.

## 2 MATERIALS AND METHODS

### 2.1 Material

The present survey has utilized a specimen of the Cameroon dwarf gecko *Lygodactylus conraui* Tornier, 1902 of about 5 cm in total length that was purchased in a pet shop and that accidentally died in captivity. The animal was immediately preserved in 80% ethanol until use. In order to systematically analyze the epidermal surface in different representative areas of the skin we have utilized small skin pieces (2–3 × 4–5 mm) that were sampled from various dorsal and ventral areas of the head, trunk, limbs, digits, and tail (mid level and near the apex) that were pre-pared and analyzed under Scanning Electron Microscopy (SEM; Figure 1). Small pieces of about 2 mm of the termi-nal region of the tail were also immediately collected post-mortem, fixed in formaldehyde and prepared for Light (LM) and Transmission Electron Microscopy (TEM).

The tail tip of *L. capensis* (Smith, 1849) was collected and prepared in the same way as above, as detailed in a previous study (Alibardi & Bonfitto, 2019). Briefly, the tail tip (bearing tail setae) was obtained after inducing autotomy, by pinching the tail (normal or regenerated) that was rapidly released by autotomy (self-amputation of the tail) and immediately fixed.

### 2.2 Methods

For scanning electron microcopy (SEM) analysis, the skin samples were dried at room temperature, and then attached to small metallic stubs (0.5–0.8 cm diameter) using double-sticky tape. They 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 18 kV.

For light and transmission electron microscopy (TEM) analysis, small pieces of the terminal region of the tail were also fixed for about 10 hr in 5% formaldehyde in 0.1 M phosphate buffer at neutral pH, dehydrated in ethanol and embedded in Bioacryl resin (Scala et al., 1992). After sectioning under an ultramicrotome (LKB, Nova), semithin sections (1–3 μm thick) were collected on glass slides and stained with 0.5% Toluidine blue for general histology or prepared for immuno-fluorescence. From areas of interest, semithin sections of 2–4 μm were attached to glass slides and dried on a hot plate. The sections were immunolabeled with the pre-core box antibody that reveals Corneous Beta Proteins (CBPs), as previously described (Alibardi & Bonfitto, 2019). The immunolabeling was revealed with fluoresceine thiocyanate (FITC, green) while nuclei were counterstained using propidium iodide (PI, red) diluted 1:100 in buffer for 10 min in the dark. Also thin sections (60–90 nm thick) were collected using an ultramicrotome on nickel grids, and they underwent immunogold stain-ing for detecting CBPs, as previously reported (Alibardi & Bonfitto, 2019). In control sections, the CBPs-antibody was omitted in the incubating solution. After labeling the sections were slightly stained in 2% uranyl acetate, dried and observed under a Zeiss 10C/CR transmission elec-tron microscope operating at 60 kV.

## 3 RESULTS

### 3.1 General shape of scales in different skin regions

In Figure 1 are indicated the 12 different skin areas, subdi-vided into dorsal and ventral sides, that were examined in the present study. In the surrounding SEM panels of Figure 1, the gross appearance of scales and their sensory organs are illustrated for each examined area. The same areas were also observed by scanning the entire surface from a low magnification to a high and detailed resolution. In order to obtain a better view of some details, the orien-tation of the specimen was varied. For each area (tail tip ventral, mid-tail ventral and dorsal, limbs dorsal and ven-tral, digits dorsal and ventral, mid-dorsum, mid-ventral trunk, nape, throat-gular and forehead) we have counted several details as reported in Table 1, but the study is how-ever mainly qualitative. Circular sensory organs, spinu-lated or flat, are generally more frequent in the caudal than the ventral aspect, and more frequent in the tail and digits than in the mid trunk and head-gular regions.

### 3.2 Micro-ornamentation of the tail tip (ventral)

Scales in the tail (Figure 1a–c), as well as in the other skin areas, consist in an external corneous beta-layer that

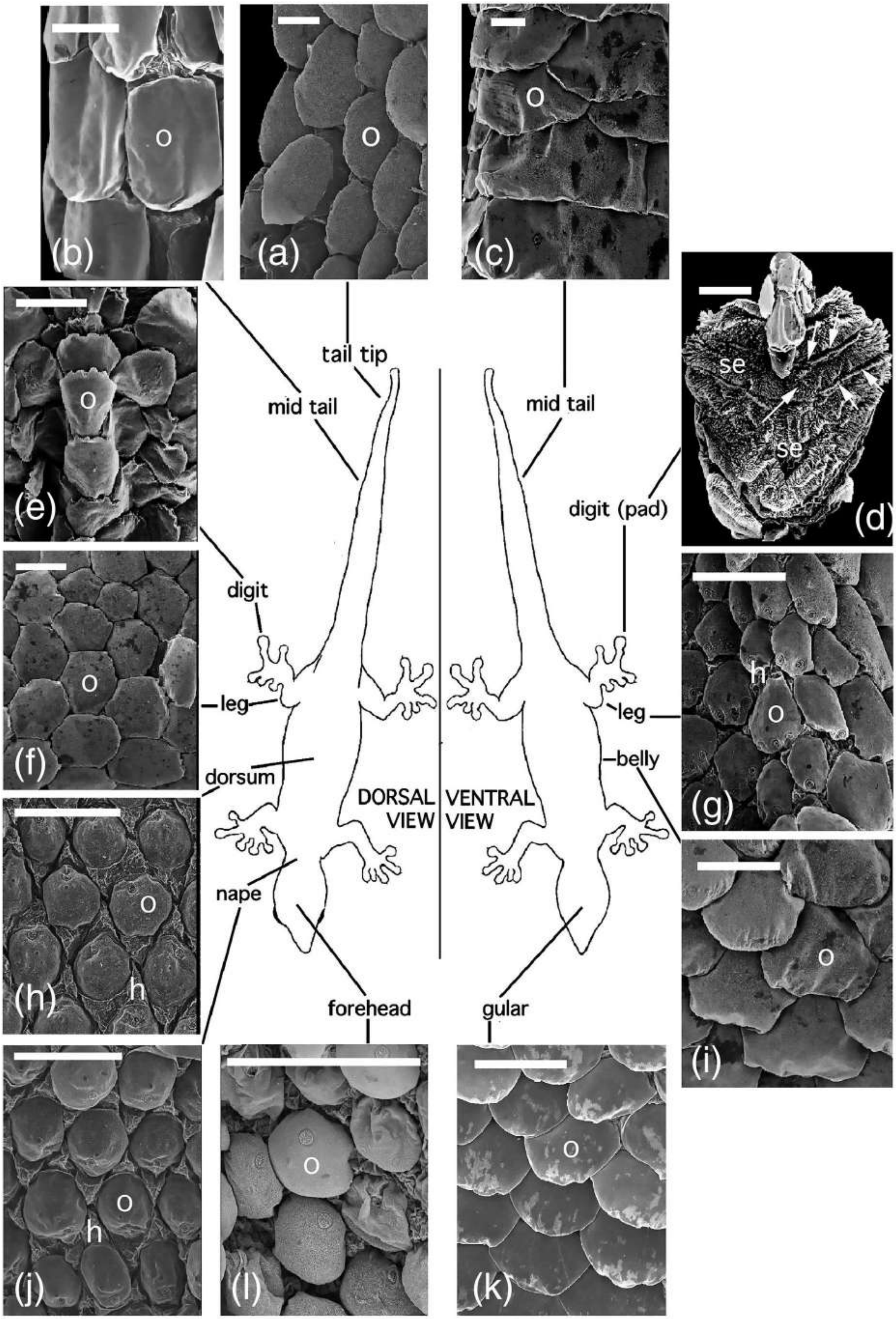

**FIGURE 1** Legend on next page.

**TABLE 1** Main characteristics of the micro ornamentation present in the skin of different areas

| Area | Scale shape | Scales ($mm^2$) | Spine length (μm) | Spinulated sensory organs | Spinulated pattern | Interscale |
|---|---|---|---|---|---|---|
| Tail tip (ventral) | Rectangular | 20 | 1 4 | 3 5 | Much prevalent | Small (overlapped) tuberculated |
| Mid tail (dorsal) | Ovoidal | 17 | 2 | 2 4 | Much prevalent | Scarce (poorly overlapped) |
| Mid tail (ventral) | Rectangular semicircular | 4 | 2 | 1 2 | Less prevalent | Scarce (overlapped) |
| Hindlimb (dorsal ventral) | Polygonal | 25 | 1 3 | 2 3 | Less prevalent | Large (not overlapped) tuberculated |
| Digit | Rhomboidal rectangular | 15 | 3 | 4 5 | Less prevalent | Small (overlapped) |
| Back (dorsum) | Ovoidal | 50 | 0.5 1 | 0 1 | Less prevalent | Large (not overlapped) |
| Belly (abdomen) | Square semicircular | 20 | 1 2 | 3 5 | Much prevalent | Small (overlapped) |
| Nape (nuchal) | Roundish | 50 | 0.5 1 | 0 1 | Less prevalent | Small (not overlapped) |
| Throat (gular) | Ovoidal | 20 | 1 3 | 1 2 | Less prevalent | Hidden but large (overlapped) |
| Forehead | Roundish | 50 | 1 3 | 1 | Less prevalent | Large (not overlapped) tuberculated |

is immunolabeled with the pre-core box anti-beta antibody, and an incomplete alpha-layer (colored inset in Figure 2a). Transmission electron microscopy shows that most immunolabeling is present in the beta-layer that is merged with the poorly labeled and superficial Oberhautchen that features the typical spinulae (Figure 2a). Denser small bundles of corneous beta material connect the beta-layer with the base of spinulae (arrowheads in Figure 2a). Underneath the compact beta-layer no gold particles are seen over the thin mesos- and alpha-cells. The SEM observation (see below) integrates this two-dimensional information from TEM, revealing a more complex picture of the Oberhautchen micro-ornamentation.

SEM observation of tail scales of 0.15–0.3 by 0.4 mm or larger on the ventral side of the tail (Figure 1c) shows that they are mainly rectangular or ovoid-like with a medium to low density and are relatively rich in sensory organs (Table 1). On the outer (dorsal) scale surface of scales, spines form a uniform bed with variable dimension, 0.5–4 μm, and few irregular patches of likely corneous material not yet lost after shedding is also observed (Figure 2b–d). At low magnification the single spinulae form the classical "spinulated pattern" of geckos but the closer observation evidences numerous atypical spinulae, a common observation also noted in other skin areas. These atypical spines actually derive from the confluence of 2–5 corneous struts (roots) 70–90 nm thick each that merge into a pointed structure forming the spine (Figure 2c,d). The observation at various angles of the spines reveals that the thin struts meet in a central axial point that appears as a "star-shaped micro-ornamentation" when viewed from above (arrowhead in Figure 2c). The complete transition in shape of these atypical spines is seen along different angles but the struts originate from a relatively smooth material that likely represents the compact beta-layer merged underneath with the Oberhautchen (Figure 2d and inset). Therefore the variation of shape and length of spinulae observed under TEM (Figure 2a) reflects this irregular 3D

**FIGURE 1** Schematic summary showing the distribution of all the areas studied on the gecko body. For each dorsal and ventral area, a low SEM image shows the general scale pattern. (a) See details in Figures 2a d and 3a d,h. (b) See details in Figures 3e,f and 4a,b. (c) See details in Figure 3g,h. (d) See details in Figure 7a g. (e) Digital dorsal scales. (f) See details in Figures 6e,f,h,i and 10e,g. (g) See details in Figure 6d,g. (h) See details in Figures 8a e and 10f. (i) See details in Figures 6a c and 8f h. (j) See details in Figure 9a c. (k) See details in Figure 9d f. (l) See details in Figure 10a d. h, hinge region (inter scale); o, outer (dorsal) scale surface; se, setae (arrows indicate the outline of a pad lamella). Bars in all figures indicate 200 μm

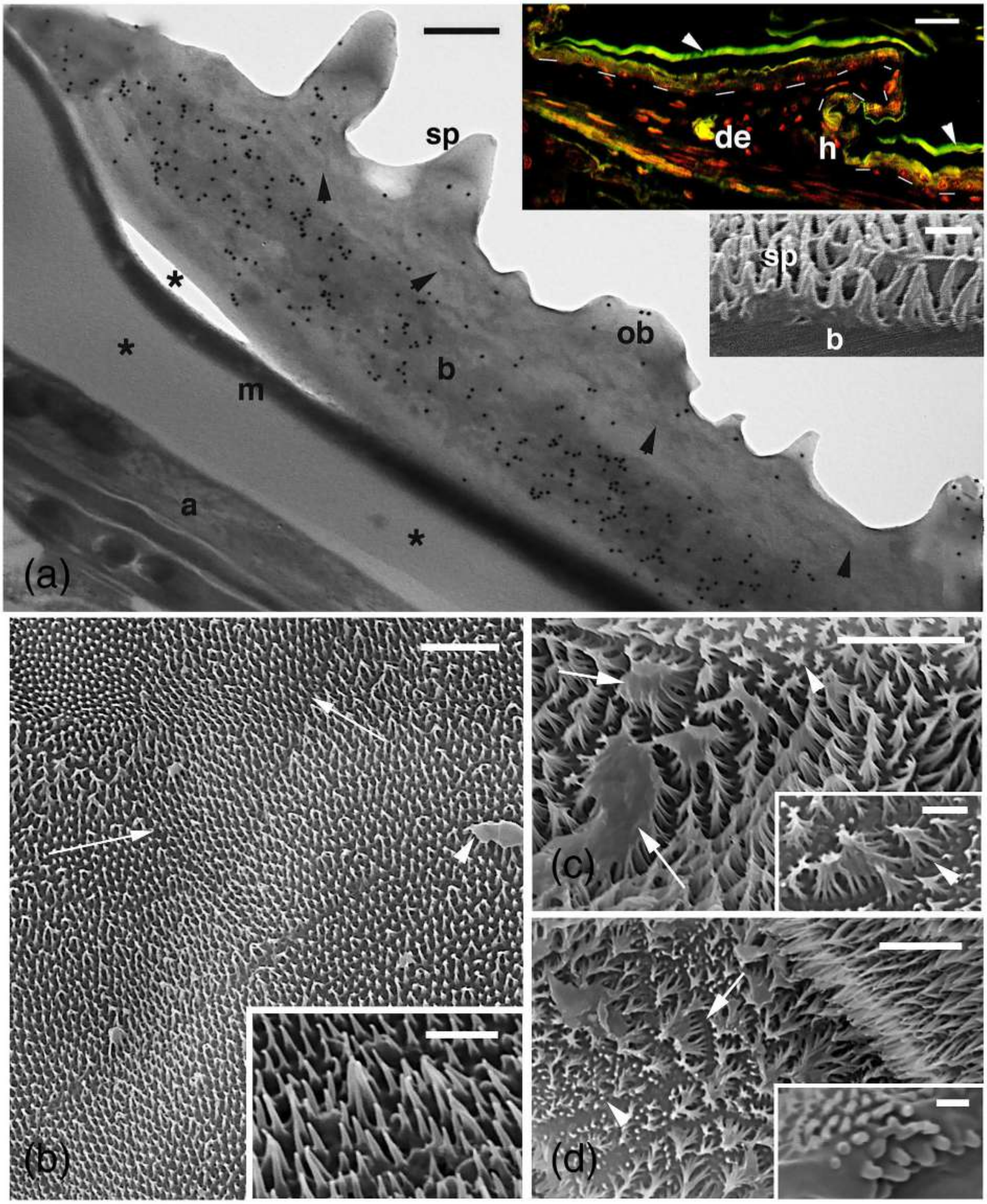

**FIGURE 2** Legend on next page.

microstructure of the "spinulae" and their forming corneous struts.

Another frequent variation of the "spinulae" in tail scales (Figure 1a–c), also noted in other areas of the skin, is that their tips in numerous areas are not separated but instead appear clumped together forming structures here indicated as "corneous belts" (Figures 2c and 3a). These belts are formed by a minimum of three to many fused spines, giving rise to a "comb-like pattern" with variable lengths (3–10 or more μm). While in some cases the tips of the atypical spinulae appear still connected to corneous fragments of the likely "unshed clear layer"

(Figures 2c and 3b), in most cases the corneous belt really forms long micro-ornamentation that does not appear as clumping artifacts (Figure 3a,c). The corneous belt or the classical spinulae disappear in rare spots or bare regions of the Oberhautchen, generally localized in the central and apical part of tail scales, and in continuation with "serpentine microridges" of corneous material of the Oberhautchen (Irish et al., 1988; sensu Maderson et al., 1998; Figure 3c,d). Thin lines forming a tile-like superficial pattern likely represent the boundary of single Oberhautchen cells (arrowheads in Figure 3d). In few and scattered areas of the outer scale surface, smooth or naked roundish regions of the Oberhautchen are present, where the spinulae terminate in forms of "roots" (Figure 3e, and inset). The specific areas occupied with corneous belts, versus areas featuring isolated spinulae, are variable in diverse scales, from an indicative average of 14% (tail scales) to 21% (forehead scales). However, in some scales the surface mainly appears covered by corneous-belts and in other mainly by separated spinulae.

The other main variation from the classical "spinulated pattern," also observed in numerous scales of the tail (Figure 1a–c), and also in other regions, is represented by the "corneous dendritic pattern" that occupies variable areas of the scales and can also terminate in exposed regions in continuation with the "serpentine microridges" (Figure 3f–h). This complex morphology results from the ramification of corneous dendritic material localized at the base of the spinulae. This complex pattern indicates that the corneous meshwork of 200–300 nm irregular filaments from which spinulae emerge from the Oberhautchen-Beta-layer can take the form of separate elements of the typical spinulated pattern or instead can form corneous belts with a "dendritic pattern" (see final drawings). The dendritic ramification indicates that the superficial spinulae are, at their base, in continuation with corneous material of the merged Oberhautchen-beta-layer, confirming the TEM analysis (Figure 2a, Alibardi, 2003, 2016). Finally, a reduction of the spine dimension is usually present in the inner (ventral) scale surface and in the inter-scale (hinge) regions where spinulae often shows a curved tip (Figures 1c and 4a,b). The latter originate from a smooth base (Oberhautchen-Beta layer) and appear with a variably folded surface that reflects the broad stretching property of the hinge region under mechanical pulling actions. The main detected sensory organs are located along the caudal border of scales (see later).

## 3.3 Tail pads in *Lygodactylus capensis*

In sparse ventral scales by the tip of the tail, groups of setae of 20–30 μm in length and 1–2 μm in diameter are present and they form adhesive pads similar to those of the digit in *L. conrau* (Table 1; Figures 1d and 4c). The setae occupy most of the distal-central area of the modified scales (lamellae) and are surrounded along the scale bor-der with much shorter spinulae with variable length (Figure 4c,d). The closer analysis of setae shows that their length decreases moving toward the border of the lamella, as in a series of progressively growing elements from the side to the center. The corneous bundles at the base of setae are in continuation with the compact Oberhautchen-beta layer present underneath, and these bundles merge into the main shaft of the setae forming a compact corne-ous rod (Figure 4e). The observation at higher magnifica-tion allows detecting the thin corneous filaments forming the setal shaft that separate into numerous secondary and tertiary branches terminating into spatula endings (Figure 4f). Aside a shorter length, tail setae in *L. capensis* are very similar to the digital setae in *L. conraui*.

After immunohistochemistry using the pre-core box antibody for CBPs, the caudal setae appear immunofluorescent (inset in Figure 5a). Under TEM-immunogold

**FIGURE 2** TEM ((a) *L. capensis*) and SEM ((b d) *L. conraui*) images of tail scales. (a) Detail of the superficial epidermal Oberhautchen beta layer that is exclusively immunolabeled for CBPs (see also the arrowheads on the green immune fluorescent beta layer in the upper inset. Nuclei are red and dashes underline the epidermis, bar, 10 μm). While the beta layer is highly labeled, the superficial Oberhautchen and spinulae are low labeled, while the meso alpha layers are unlabeled (asterisks indicate artifact layer separation). Arrowheads indicate compacted bundles of corneous material of merged beta cells that insert into the spinulae. Bar, 0.25 μm. The lower inset (bar, 1 μm) shows a SEM view of Oberhautchen spinulae merged with the beta layer. (b) Uniform spinulated pattern presents in a scale located at the tail tip. Lines (arrows) indicate cell boundaries of Oberhautchen cells. The arrowhead points a cell fragment, likely derived from the clear layer. Bar, 4 μm. Inset (bar, 3 μm) shows a detail of the spines. (c) Area of outer scale surface showing "composed" spinulae made by aggregated filaments (roots or thin struts) and clear layer remnants (arrow). The arrowhead indicates a composed spinula observed in dorsal view. Bar, 10 μm. The inset (bar, 2 μm) shows the aggregated roots (arrowhead) forming the composite spinulae. (d) Other region featuring numerous composite spinulae (arrow) that progressively turn in tiny spines (arrowhead) in the lower area. Bar, 10 μm. The inset (bar, 1 μm) details the spinulae reduction in size. a, alpha cells; b, beta layer; de, dermis; h, hinge region; m, mesos cells; ob, Oberhautchen with spinulae; sp, spinulae

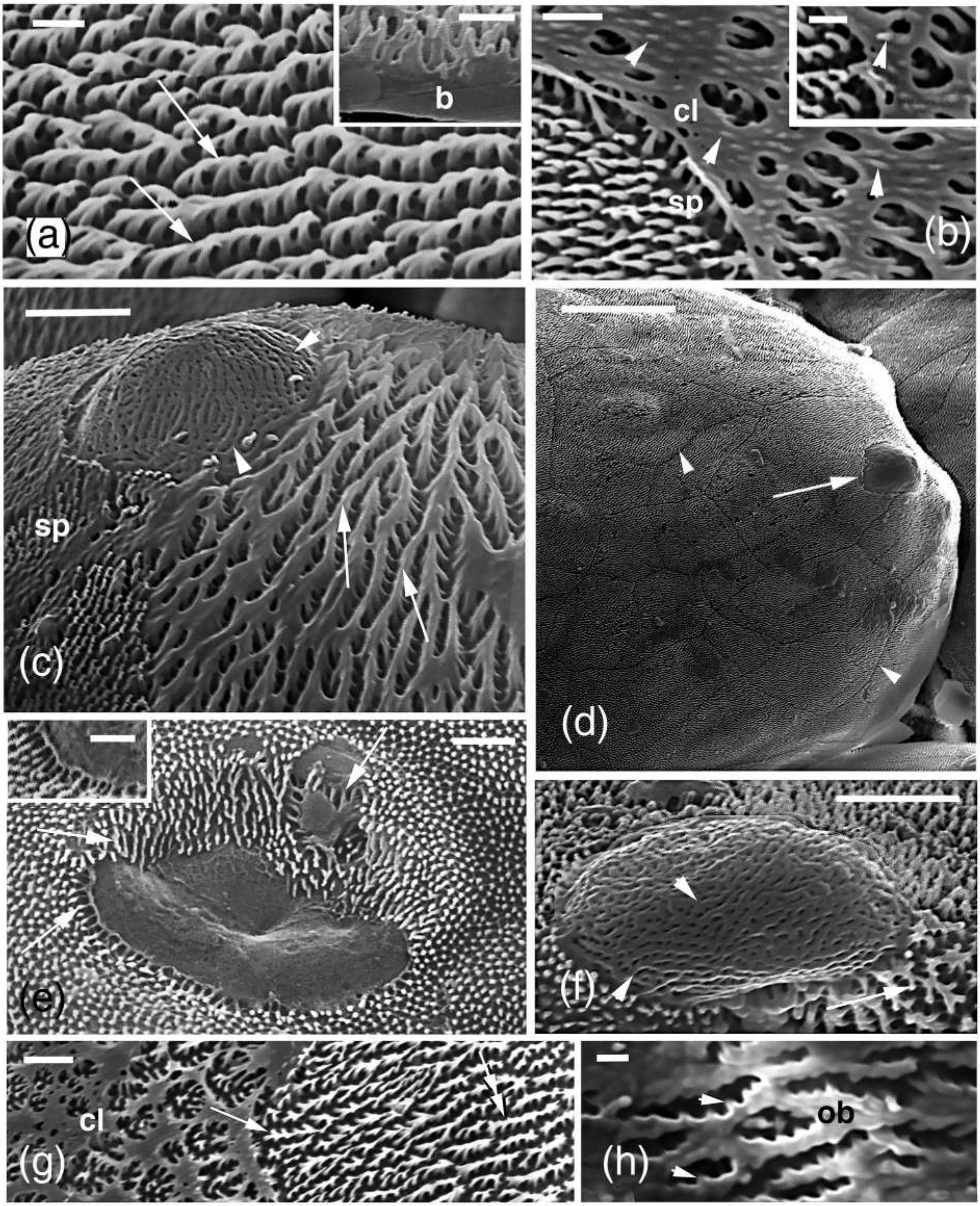

**FIGURE 3** Legend on next page.

labeling the bundles of corneous beta-material accumulating at the base of the forming setae also appear labeled (arrowheads in Figure 5a). These bundles correspond to the corneous struts observed under SEM that agglutinate and give rise to the spines (Figure 2c,d). Around the forming setae, denser fibrous material contained in the cytoplasm of clear cells is present but this is unlabeled for CBPs (arrows in Figure 5a). Also the mature outer setae resting upon the compact Oberhautchen-beta-layer are immunolabeled, while the thin mesos and alpha layers located underneath the beta-layer are not labeled (Figure 5b).

## 3.4 Micro-ornamentation of the dorsal and ventral mid-tail

The dorsal scales in this region are generally smaller (0.15–0.2 by 0.3–0.35 mm) then those in the ventral position (0.5 mm or larger), and appear more rectangular than scales located at the tail tip (Table 1; Figures 1a–c and 6a). They feature an alternate disposition and are overlapped so that the hinge (inter-scale) regions appear almost absent. Micro-ornamentation resembles those described in distal most scales of the tail and rare spots of bare areas, real or artifactual (derived from sample preparation), are seen. The latter probably represents exposed regions of compact corneous material from beta-cells. The distribution of spinulae, pointed or with a curved tip (prongs, Figure 6b) appears relatively uniform with similar distribution to those forming the corneous belts, as previously described. Large areas of the outer scale surface contain a dendritic ramification pattern as previously described (Figure 6c). These scales often show a "bare-knob" of 6–8 by 10–15 μm that is centrally localized at their caudal tip, possibly corresponding to a reduction of the Oberautchen micro-ornamentation or to a thinning of the epidermis related to some sensorial function. Some scales in this region possess 2–4 spinulated sensory organs each, and they are located toward the apical side of the scale.

On the ventral side of the tail, scales are larger than on the dorsal side and therefore less numerous per unit of area (Figure 1c; Table 1). They are mainly rectangular with the main axis perpendicular to the body axis, variably overlapped and their surface is covered with more or less uniform spinulated beds. Numerous spinulae also appear as derived from the coalescence of 3–4 thinner struts of corneous material (100–300 nm diameter), as previously described. Sensorial organs similar to those of the tail tip are less frequent than in the ventral with respect to the dorsal side in this skin area.

## 3.5 Micro-ornamentation of the hindlimb (leg) and digital pads

The observation of dorsal and ventral scales in this region shows that they have a small size (0.1–0.15 by 0.2–0.25 mm) and irregular disposition, and are generally polygonal-shaped (Figure 1e–g; Table 1). The inter-scale regions are generally visible more than in other skin areas, especially in scales of the dorsal side of the leg that are not overlapped. The numerous folds present in the small micro-ornamentation of the hinge regions indicate a broad capacity for stretching in these scales. The outer side of leg scales is more or less evenly covered by short spinulae while 2–3 large sensory organs (15–20 μm in diameter) are commonly seen located toward the apical (caudal) border of ventral scales (Table 1; Figure 6d–f). Also small, elevated and bare spots of 5–10 μm by 20–30 μm are seen toward the anterior border of these scales and also very close to the spinulated sensory organs (Figure 6f,g). The micro-ornamentation presents isolated spines and composed spines that derive from the aggregation of corneous struts and sparse corneous belts (Figure 6h). An elaborated micro-ornamentation forming long or short dendritic ramifications is typically located in these scales, testimonial of a complex basal meshwork of corneous material in continuation with beta-cells and from which spinulated protrusions (spines) are originated in the Oberhautchen, as it is visible in other areas of the scale (Figure 6i, see Section 4).

On the ventral side of the digits is present the adhesive pad (Figure 1d). This is formed by a number of distinctive overlapped lamellae (modified scales) covered with setae of 30–60 μm in length and 1.5–3 μm in diameter at their base (Figures 1d and 7a). The apical (distal) part of each lamella contains most of the setae and in particular the longer ones of 50–60 μm in length. The setae are organized in a palisade at the anterior and lat-eral borders of the outer lamella surface while on the

**FIGURE 3** SEM images of tail scales in *L. conraui*. (a) Detail on a small area of a scale that is located near the apex of the tail showing the corneous belts (arrows) bonded to spinulae tips. Bar, 2 μm. The inset (bar, 2 μm) shows the spinulae merged with the beta layer. (b) Detail of other area of tail outer surface with numerous spinulae still inserted with their tips (arrowheads) to fragments of un detached clear layer. Bar, 2 μm. The inset (bar, 1 μm) detail on an inserted spinula tip (arrowhead) in a fragment of the clear layer. (c) Apical area of a mid tail dorsal scale featuring an Oberhautchen dome organ made by "serpentine microridges" (arrowheads). The spinulae turn into a parallel series of corneous belts (arrows) that are visible on the right. Bar, 5 μm. (d) Broader view of a dorsal scale showing an apical bare region (arrow) with a spinulated surface. Cell boundaries are visible (arrowheads). Bar, 20 μm. (e) Detail on a smooth area (naked Oberhautchen) in continuation through roots (arrows) with the surrounding spinulated areas. Bar, 3 μm. The inset (bar, 1 μm) is a close up view of the roots. (f) detail on a curved "serpentine microridges" (arrowheads) forming an oval area surrounded with spinulae and "corneous dendritic ramification" (arrow) of the Oberhautchen. Bar, 4 μm. (g) Dorsal view of an area of very apical tail scale showing an apparent "corneous dendritic patter" (arrow) mixed with rows of spinulae that are aggregated into "corneous belts" (double arrow). Bar, 4 μm. (h) Detail on a "dendritic pattern" of the Oberhautchen that is likely observed from below (arrowheads point to spinulae). Bar, 1 μm. b, compact beta layer; cl, clear layer remnant; ob, Oberhautchen; sp, spinulae

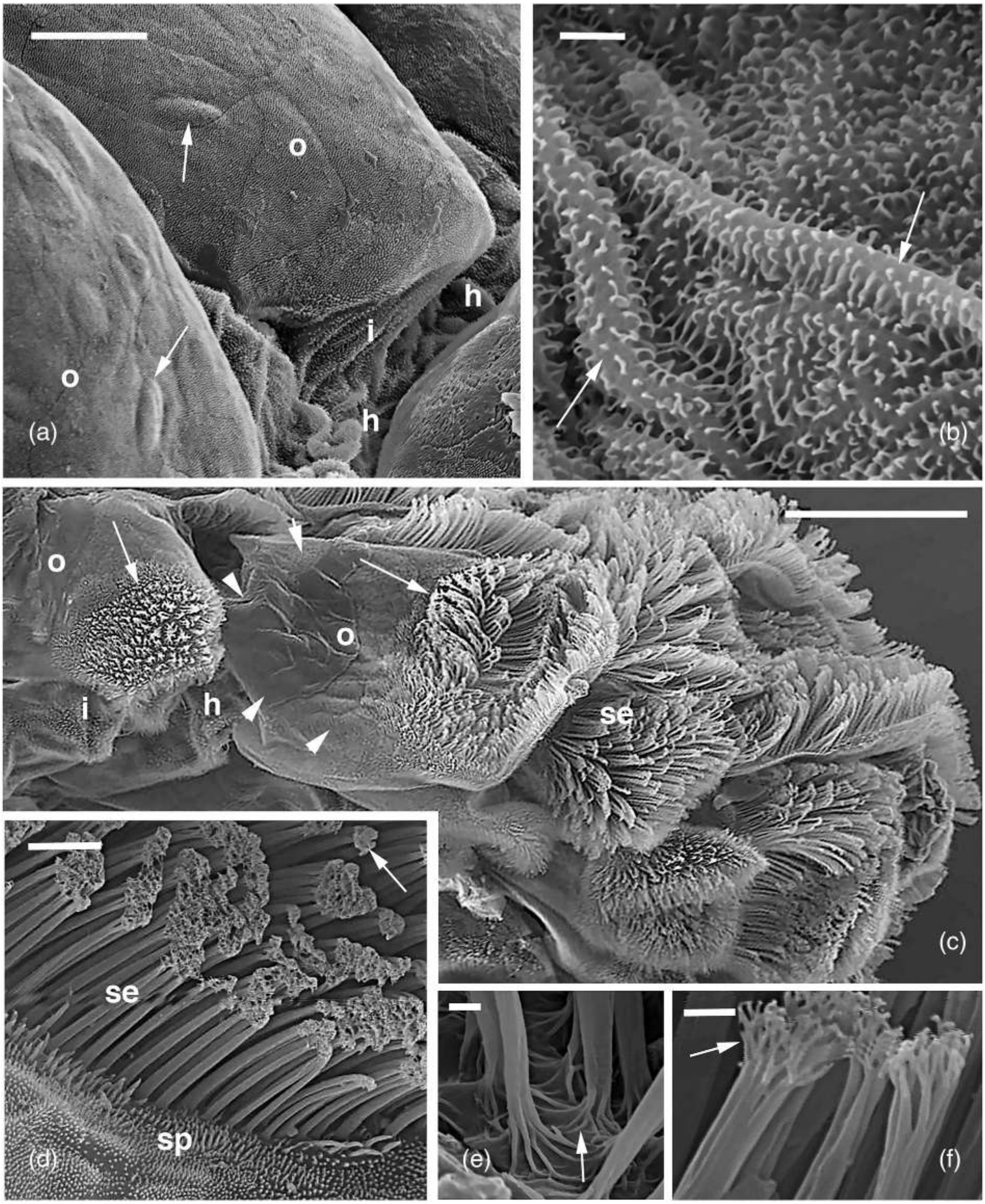

**FIGURE 4** SEM views of mid tail dorsal scales ((a, b) from *L. conraui*) and pad lamellae with setae from apical tail (from *L. capensis* (c f)). (a) General aspect of outer and inner scale surfaces observed from the side. Arrows indicate oval curved spots of the Oberhautchen. Numerous epidermal folds covered by a bed of fine spinulae are observed in the hinge region. Bar, 20 μm. (b) Detail of the separated, short and curved spinulae present in the hinge region. Arrows indicate folds. Bar, 2 μm. (c) Rows of tail setae featuring the spinulated basal part of the lamellae (arrowheads) and the apical region occupied with setae (arrows). Bar, 100 μm. (d) Detail on groups of setae (arrows on the apical ends) outlined by spinulae along the lamella border. Bar, 10 μm. (e) Detail of base of the setae formed by the fusion of corneous roots (arrow). Bar, 2 μm. (f) Apex of some setae showing their branched tips (arrow). Bar, 2 μm. h, hinge region (interscale); i, inner scale/lamella surface; o, outer scale/lamella surface; se, setae; sp, spinulae

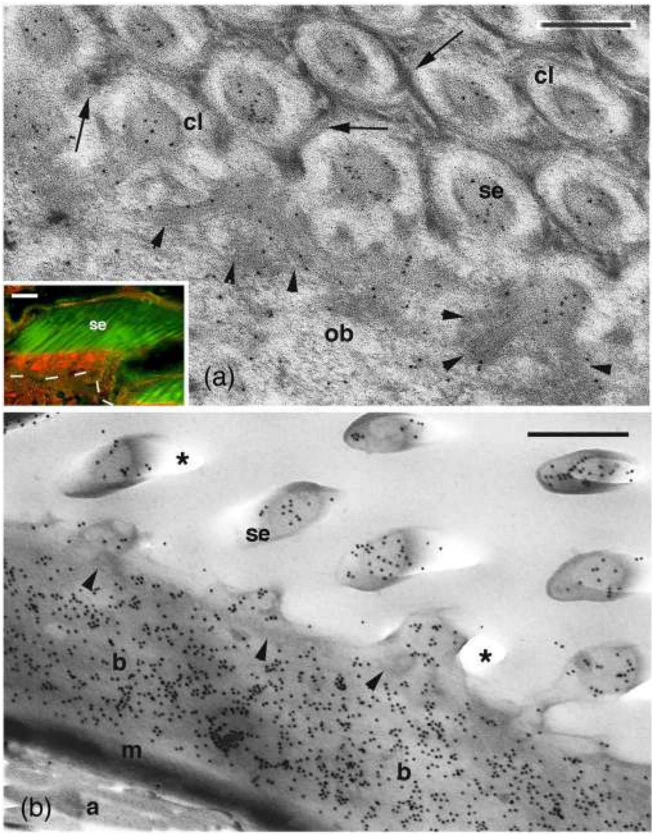

**FIGURE 5** LM (inset) and TEM immunogold labeling (a, b) for CBPs in tail adhesive setae of *L. capensis*. (a) Close up in forming setae of the inner generation (indicated as green immunofluorescent in the inset while nuclei are red and dashes underline the epidermis; bar, 10 μm). Arrowheads indicate the labeled corneous beta bundles forming the base of the setae. Arrows point to the fibrous belts of the clear cells that surround the forming setae. Bar, 0.5 μm. (b) Immunolabeled mature setae and beta layer of the outer setal generation, showing small corneous bundles (arrowheads) at the base of setae. Asterisks indicate sectioning artifacts. Bar, 0.5 μm. a, alpha cells; b, beta layer; cl, clear cell (cytoplasm); m, mesos cell; ob, Oberhautchen; se, setae (in cross oblique section)

inner surface setae are replaced by spinulae (Figure 7a–c). The perimeter of the lamellae is bordered with short spinulae but toward the hinge region a gradation of setae into spinulae is visible. The structure of digital setae of *L. conraui* resembles those of the tail in *L. capensis* although digital setae are longer. Digital setae also appear to originate from the aggregation of corneous filaments (roots) in continuation with the Oberhautchen-beta layer (Figure 7d). The length of setae varies and moving more proximally, toward the hinge region that is normally covered by the more proximal next lamella, becomes half or less of the length of the distal setae (Figure 7e). Longer or shorter setae however branch profusely near their tip giving off numerous thinner stems that terminate into spatulae (Figure 7e,f). The examination at high magnification shows the filamentous meshwork of paler filaments of corneous beta-material separated by denser inter-filaments regions in the setae (Figure 7g). On the dorsal side the digits show small rectangular to oval-shaped scales (Figure 1e) with a similar micro-ornamentation as previously reported for the mid-tail areas.

## 3.6 | Micro-ornamentation of the dorsal trunk (back)

The scales appear numerous and generally small (0.08–0.1 by 0.08–0.1 mm), with an oval shape and broad inter-scale (hinge) areas (Figure 1h; Table 1). The surface of the hinge region appears wrinkled and occupied with numerous folds suggesting that inter-scale regions can be stretched broadly under mechanical tensions on the skin (Figure 8a,b). The surface of the outer scale features mixed area of spinulae and corneous dendritic ramifications of the Oberhautchen (inset in Figure 8a). Also, the surface presents sparse and small oval-shaped bumps of 5–8 μm, derived from local elevations of the Oberhautchen surface (arrows in Figure 8a,b). The examination at higher magnification of these small regions shows that the spinulae lose their identity and take a corneous reticulate pattern, indicating that these spots represent transitional zones where the Oberhautchen micro-ornamentation changes pattern (Figure 8c). The hinge region instead presents short, less than 0.5 μm in length, spinulae or a prevalent "corneous dendritic pattern," made of short ramifications (inset of Figure 8b).

A peculiar characteristic of these scales is the presence of a large spinulated sensory organ located at the distal (caudal) scale surface and of a bouton-like smooth area seen in the central and more elevated point of the scale (arrows in Figure 8a,b,d). At higher magnification this spot appears as a dome-like and naked Oberhautchen area of 6–8 by 5–8 by 5–7 (high) μm showing a "serpentine microridge pattern" (sensu Maderson et al., 1998; inset in Figure 8d). The spinulated sensory organs resemble those previously described in other skin areas but contain a long central bristle emerging from the surrounding spinulae (see later description). This bare scale organ may represent a possible tactile organ or a thinner region of the epidermis that can detect some sensory stimulation (see Harvey & Gutberlet, 1995). The perimeter of single Oberhautchen cells is often visible as a darker line enclosing a polygonal-shaped cell, but the higher magnification examination reveals that these lines result from the narrow interruption (0.2–0.4 μm

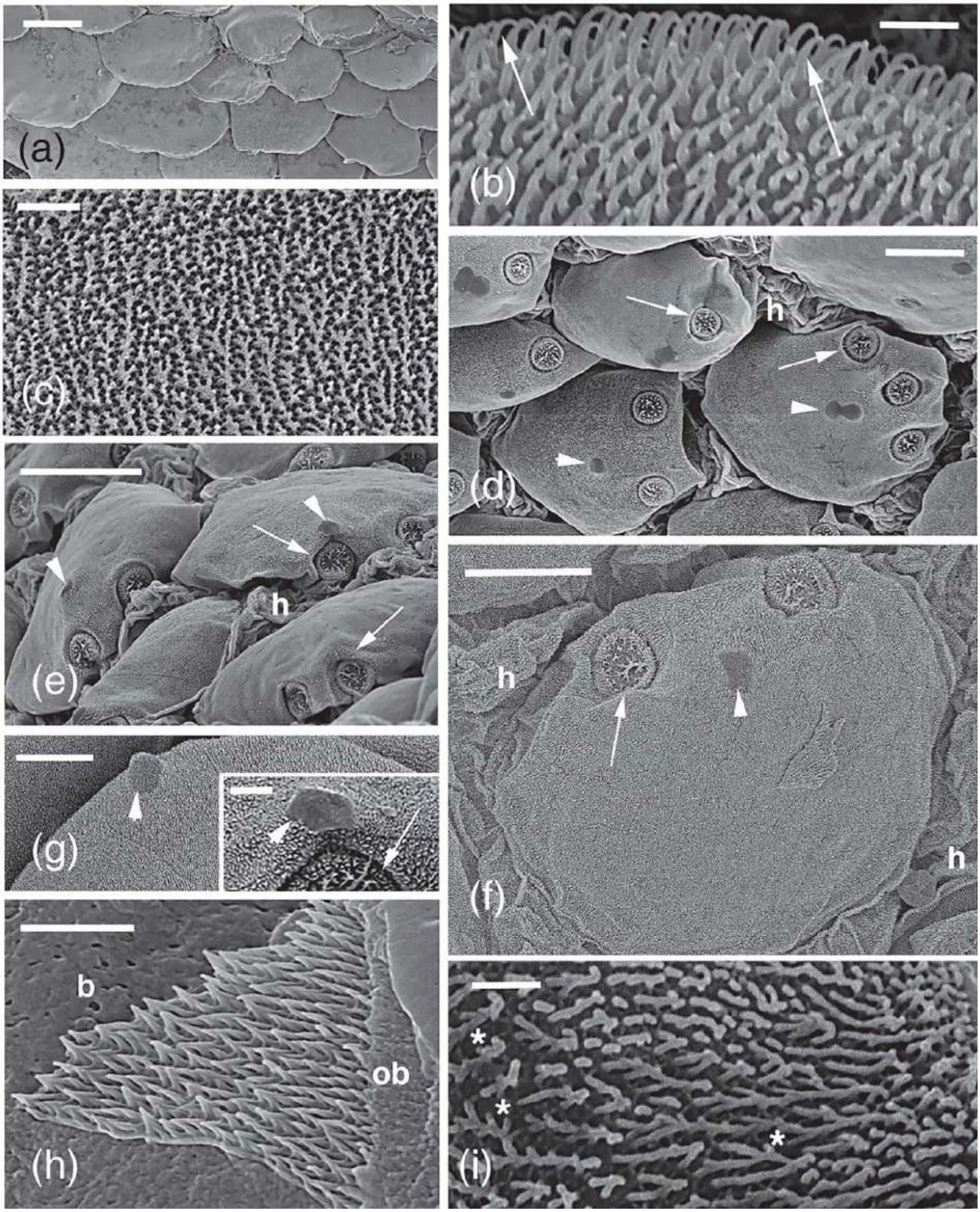

**FIGURE 6** Legend on next page.

broad) of the continuity of the spinulae that otherwise remain connected by thin corneous struts or roots of 80–100 nm (Figure 8e). Five to six struts starting at the base of the spinulae converge at the base of the single spinulae and appear like a stars-like image when observed from above.

## 3.7 Micro-ornamentation of the ventral trunk (belly/abdomen)

Numerous overlapped scales of medium to small dimension (0.1--0.2 by 0.2–0.3 mm; Figures 1i and 8f; Table 1) and ovoidal to rectangular in shape are evenly coated with isolated spinulae alternated with areas of spinulae connected at their apex and forming comb-like "corneous belts" of variable length (3–10 μm; Figure 8g; Table 1). The most hidden inner scale surfaces and hinge regions are covered with numerous but very short and separated spinulae, emerging from a smooth Oberhautchen surface, and often featuring a bent hook, resembling small prongs (Figure 8h). The number of spinulated sensory organs, localized by the distal apex of ventral scales, varies on different scales.

## 3.8 | Micro-ornamentation of the nape (nuchal region)

This region is composed of small circular to oval scales (0.08–0.1 by 0.1–0.1 mm) with broad inter-scale areas, resembling also in dimension those of the back (Figures 1j and 9a; Table 1). The outer scale surface is covered by numerous spinulae beds interrupted in small areas by ramified dendritic corneous patterns (arrow in Figure 9b). The central region toward the distal part of these scales is occupied by a bare-knob (scale organ like in scales of the back) while a large sensory organ with 5–6 bristles of 10–20 by 0.5–1.5 μm is present near the caudal border. Like for other bristles, also in this case they results from the basal fusion of thinner corneous roots (Figure 9c). The remaining outer and inner scale surfaces are evenly coated with very small spinulae, like in other regions.

## 3.9 Micro-ornamentation of the gular region (throat)

Numerous small and ovoidal scales (0.08–0.15 by 0.1–0.2 mm) with irregular caudal border are present and they appear slightly overlapped (Figures 1k and 9d,e). A dorsal view of this area shows an apparently reduced inter-scale region, but both the inner surface and hinge regions are better viewed when observed from the side or tilting the specimen (Figure 9f). The latter shows that numerous scales in this region are almost vertically oriented, but this condition maybe is artifactual, following fixation. The inner surface and hinge region appear very folded, indicating that gular scales can broadly stretch under mechanic stress. The outer scale surface is covered by an even stratum of spinulae of 1 μm in length that reach 3 μm on the dorsal side of these scales, and few other micro-ornamentation patterns are seen.

In the folded inner scale surface and in hinge regions the Oberhautchen pattern is altered and spines are blunted, isolated or forming a "serpentine microridge pat-tern" (insets in Figure 9f). Here the micro-ornamentation pattern transits from single, short and blunted spinulae to elongated worm-like forms of corneous material that may reflect the orientation that the corneous material takes during its accumulation in these cells and that facilitate stretching. Like or even more frequently than in other skin regions, small corneous fragments are present, possibly representing dirt (fragments from preparation) or true debris derived from a noncompletely shed clear layer.

## 3.10 Micro-ornamentation of the forehead

Also here numerous small scales with roundish to oval shape (0.08–0.1 by 0.1–0.5 mm, Table 1) are present, with relatively wide inter-scale areas showing numerous folds (Figures 1l and 10a). The outer surface of scales is cov-ered with a prevalent spinulae bed but also sparse areas occupied by the reticulate dendritic corneous patters are present (Figure 10b). One large spiny sensory organ with a single long bristle of over 10 μm occupies a more cen-tral area of the scale. In addition, a bare-knob with a "serpentine micro-ornamentation" or even a flat and lenticular tactile organ of 5–6 by 6–8 μm is centered in the scale near the sensory organ (see later description). The numerous wrinkles present on the inner scale sur-face but especially inter-scale region also suggest an

**FIGURE 6** SEM view of mid trunk ventral scales (a c) and limb scales (d i) of *L. conraui*. (a) Low magnification view that shows the size variations. Bar, 200 μm. (b) High magnification view evidencing the hooked shape (arrows) of numerous spinulae (small prongs). Bar, 2 μm. (c) Area of the outer scale surface occupied by a prevalent "corneous ramificated pattern." Bar, 4 μm. (d) Oval shaped scales with numerous spinulated sensory organs (arrows) close to "naked areas" (arrowheads). The latter possibly represent other sensorial regions. Bar 40 μm. (e) Limb scales seen in frontal view, and evidencing the naked (arrowheads) and spinulated (arrows) sensory organs. Bar, 50 μm. (f) Dorsal view of limb scale with an irregular naked area (arrowhead) and two large spiny sensory organs (arrow). Bar, 30 μm. (g) Anterior margin of the outer scale surface of ventral limb scale with a protruding naked organ (arrowhead). Bar, 20 μm. The inset (bar, 4 μm) indicates the close position between a naked area (arrowhead) and a spinulated sensory organ (arrow). (h) Detail at high magnification on the Oberhautchen surface showing the stratification and its continuity with the underlying beta layer. Bar, 4 μm. (i) Detail of the dendritic ramification pattern in the outer scale surface of a limb area. The dark regions (asterisks) represent the base of the Oberhautchen layer bar, 2 μm. b, pitted beta layer; h, hinge region; ob, Oberhautchen

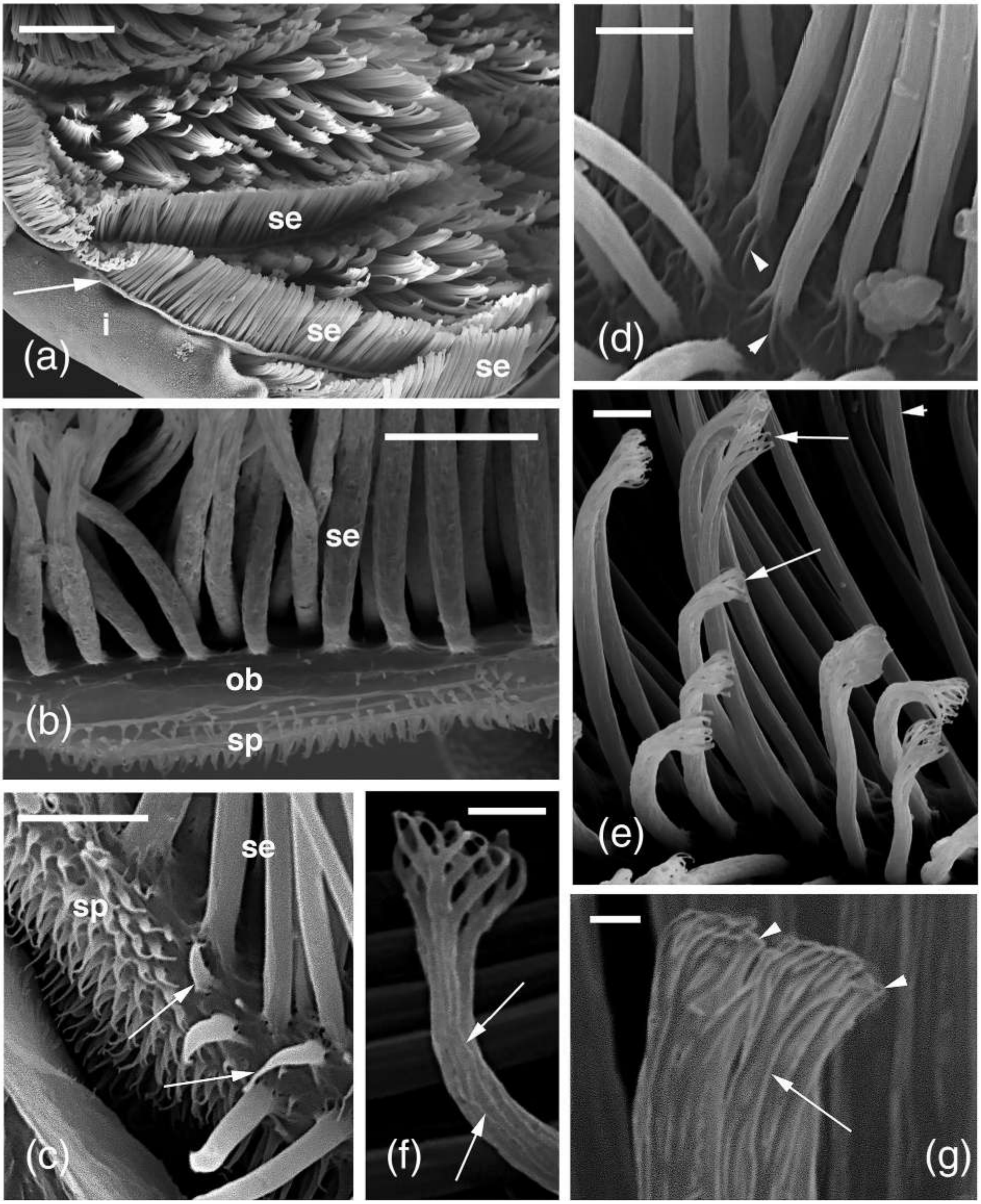

**FIGURE 7** SEM images from the digital pads of *L. conraui*. (a) Overlapped pad lamellae with their apical/distal setae. The arrow indicates the lateral border of a lamella. Bar, 50 μm. (b) Front view of a lamella with the apical row of setae localized on the outer lamella surface and the spinulae in the apical inner lamella surface. Bar, 10 μm. (c) Detail of the lateral border of a pad lamella that shows spines (arrows) with intermediate sizes between spinulae and setae. Bar, 5 μm. (d) Detail on the basal part of some setae that reveals the confluence of corneous roots/struts (arrowheads) that form the setae. Bar 5 μm. (e) Setae with apical branching (arrows) showing different lengths from the proximal side of the lamella toward the longer and more apical setae (arrowhead). Bar, 4 μm. (f) Detail on the intricate branching of a seta where some axial corneous filaments (arrows) are observed in continuation with the basal roots. Bar, 2 μm. (g) High magnification detail of the apical portion in a seta branching showing the corneous filaments (arrow) and the terminal spatulae (arrowheads). Bar, 1 μm. i, inner lamella surface; ob, Oberhautchen (marginal/anterior); se, setae; sp, spinulae

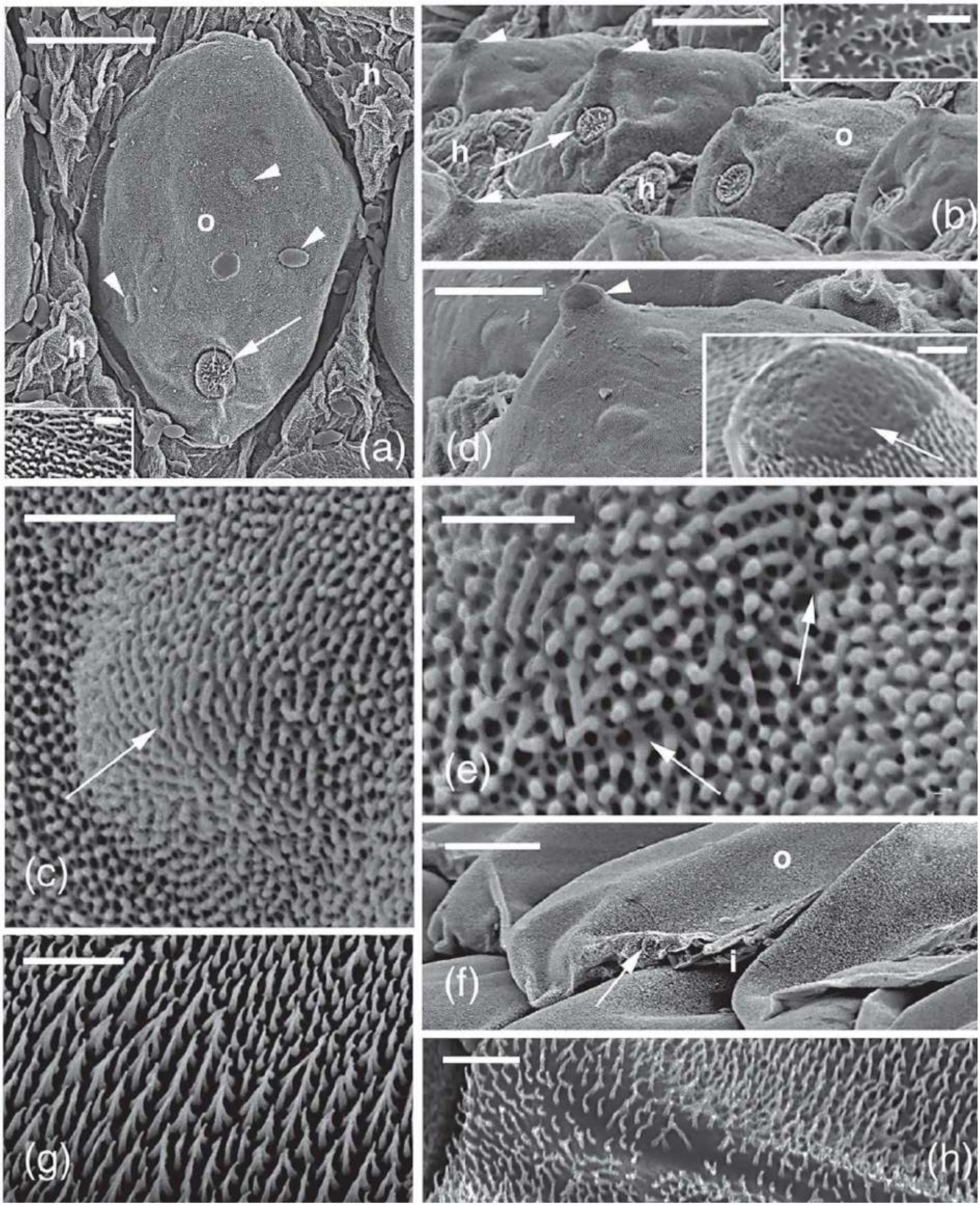

**FIGURE 8** Legend on next page.

ample stretching capability in the head skin. At higher magnification, the surface of the inter-scale region appears covered by separated tiny spinulae of 0.2–0.5 μm (Figure 10c). Their shape varies from pointed with a curved tip to a blunt tip that terminated into a flat corneous material of the Oberhautchen layer.

## 3.11 Sensory organs

Most sensory organs of 10–20 μm in diameter are recorded in the distal (caudally directed) and front part of scales in every skin areas here examined. They are of the spinulated type while bouton-like and flat tactile organs (Ananjeva & Matveyeva-Dujsebayeva, 1996; Sherbrooke & Nagle, 1996) are not surely detected in this species. Other sensorial regions are represented by the flat or domed areas, showing a smooth or a "serpen-tine-microridge pattern" (Figures 3e,f, 6g, 8d and 10d; sensu Maderson et al., 1998). Table 1 resumes the num-ber of the spinulated sensory organs present over each scale type. The spiny organs (setae bearing sensilla, sensu Hiller, 1977) consist of an encircling groove con-taining a round disk covered with 2–4 μm long single or confluent spinules (composed by 3–5 corneous struts) that become 3–5 longer bristles of 8–12 μm in length in the central part of the organ (Figures 9c and 10d–g). The circular rim contains a thin pellicle inside, likely corre-sponding to the thin Oberhautchen surrounding this sensory organ but also corneous roots are observed, and they are connected with the surrounding spinulae of the Oberhautchen (Figure 10f,g). In some preparations the circular groove appears stretched and smooth revealing its continuity with the surrounding spinulated Ober-hautchen, as indicated by the presence of few or numer-ous corneous roots.

The spinulated micro-ornamentation mix with com-posed spinulae (by the aggregation of 4–6 thin corneous struts) or with corneous dendritic ramification, which density decreases toward the central area occupied by longer bristles. Some bristles also evidence small branches stemming along the their shaft in addition to basal corneous roots (arrowheads in Figure 10f,g). The larger sensory organs are seen in the dorsal scales of the back (10–15 μm large), and they present a prevalent long bristle of 8–20 μm by 0.8–1.2 μm at the base that thins out and takes a hook-like shape apically. In other skin regions, 3–5 bristles of 6–15 μm length by 0.8–1 μm diameter are instead present. The latter often show short branching or even thin corneous roots of 80–100 nm thick that merge with the basal Ober-hautchen layer, as it is observed in setae of digits or tail (Figures 4e and 7d).

# 4 DISCUSSION

## 4.1 Origin of the micro-ornamentation variations

Although only one specimen of *L. conraui* was available in the present analysis, we are relatively confident that the study is representative of microstructure variations typical of the species. In fact, also in the tail scales of *L. capensis* similar variations have been observed although a body map was not made for this species (Alibardi & Bonfitto, 2019). SEM studies on lizard micro-ornamentation are generally limited only to small areas of the skin, and therefore it is unknown if the pattern detected in these few areas is representative of the entire skin. In contrast, after the examination of numerous scales in selected areas of the body (Figure 1), the present study provides a skin map of micro-ornamentation varia-tion in *L. conraui*. A more complex and variable pattern of micro-ornamentation is present in different regions of scales and comprises transitional areas where the accumulation of CBPs gives rise to different patterns (Maderson et al., 1998). The variation in micro-ornamentation reflects a real condition but we cannot exclude for some artifact modifications such as sticking of spinulae, their damage during preparation, fragments dispersion, or alteration due to a natural wearing of spinulae.

Ultrastructural studies on the formation of spinulae and other micro-ornamentation during differentiation of Oberhautchen cells have shown that small and irreg-ular beta-packets or beta-bundles containing CBPs are formed in the cytoplasm, together denser round granules of unknown composition but rich in sulfur, histidine, and proline (Alibardi, 1999, 2002, 2003, 2016; Landmann, 1979; Maderson et al., 1972, 1998; Figure 11a–c). The fusion of beta-packets gives rise to corneous bundles that originate the axial and lateral struts that sustain/compose spinulae or setae

**FIGURE 8** SEM images from mid trunk dorsal scales (a e) and mid trunk ventral scales (f h) of *L. conraui*. (a) Oval scale surrounded by broad inter scale (hinge) regions. Arrowheads indicate elevated oval spots of the Oberhautchen in the outer scale surface. The arrow points to a spinulated sensory organ. Bar, 50 μm. The inset (bar, 1 μm) shows a small area with a dendritic mixed to a spinulated pattern.
(b) Lateral view of dorsal scales that evidences the dome like shape of their outer surface where a spinulated sensillo (arrow) and an apical "naked region" (arrowheads) are present. Bar, 50 μm. The inset (bar, 1 μm) depicts the small spinulae covering the inner scale surface and extremely folded hinge regions. (c) Detail on an oval elevation of the Oberhautchen surface where also a dendritic pattern (arrow) is present. Bar, 4 μm. (d) Detail of a dorsal scale that shows a smooth area (arrowhead) that is enlarged in the inset. Bar 20 μm. The inset at higher magnification shows that the smooth organ features a typical "serpentine microridge pattern" (arrow). Bar, 2 μm. (e) High magnification view along a cell boundary (arrows) where numerous "roots" representing cell junctions or corneous filaments are present. Bar, 2 μm. (f) Overlapped mid trunk ventral scales with an apical sense organ (arrow). Bar, 50 μm. (g) Detail of a mainly spinulated Oberhautchen surface. Bar, 4 μm. (h) Separated, small and curved spinulae detected in the inner scale surface. Bar, 4 μm. h, hinge (inter scale) region; I, inner scale surface; o, outer scale surface

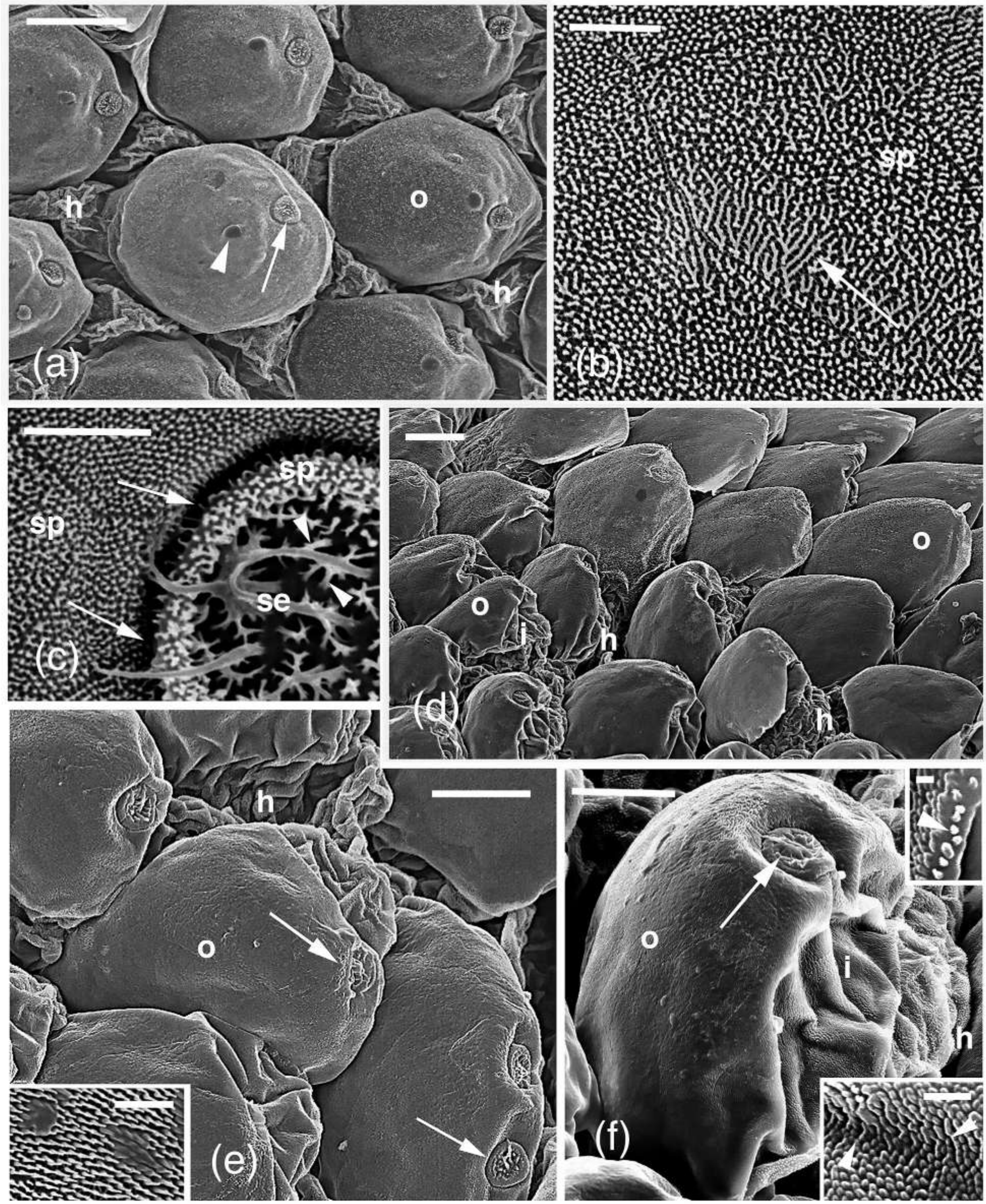

**FIGURE 9** Legend on next page.

(Figure 11d,e). These struts or roots of corneous material at the base of spinulae are also visible in other studies (Ruibal, 1968, Figures 1a,d and 2a; Stuart & Daniel, 1972, Figure 4; Hiller, 1977, Figures 6 and 7; Harvey & Gutberlet, 1995, Figure 10e,f; Irish et al., 1988, Figure 8c,e). This corneous material eventually merges with the more compact material of beta-cells so that the two layers become continuous at maturation (Figure 2a lower inset, Figure 3a inset, Figure 11c–e). The micro-ornamentation pattern on the epidermal surface is generally devoid of fragments of the clear layer but in some areas this is not the case, indicating that molt fragments may result from the process, especially in lizards (Figure 11f).

Under TEM observation the spinulated pattern appears as pointed or rounded spinulae but the SEM observations indicate that this pattern may be diversified in at least two or three others patterns here indicates as "corneous belts," "corneous dendritic ramifications" and "serpentine-microridges" (Figure 12a–c). Corneous belts appear slightly more common in the anterior scales (gular-forehead and nape) than in the remaining body areas. Variations of the "dendritic pattern" are seen in few areas of the Oberhautchen in form of a "serpentine microridge pattern" (see Fig. 13 in Maderson et al., 1998), also indicated by other authors as "pit and groove" pattern (Fig. 5F in Peterson, 1984). This aspect of the Oberhautchen likely represents areas of reduction in the deposition of corneous beta-material that gives rise to naked or smooth surface regions ("sensu" Maderson et al., 1998). We have here observed this pattern mainly in the hinge region of the gular scales (Figure 9f). The observed variations, from a smooth Oberhautchen surface, to the corneous belts or to the corneous dendritic ramification or the serpentine microridges, derive from the different pattern of accumulation of corneous beta material (formerly indicated as beta-keratin) in the Oberhautchen during scale growth. The spines forming the classical spinulated pattern of geckos and iguanid lizards, isolated or derived from the clumping of corneous struts (Figure 2b–d), represent the prevalent micro-ornamentation variation observed in most scales.

The thickness of the mature Oberhautchen at the base of the spinulae is very thin, in the range of 0.2–0.5 μm, and it is merged with the beta-layer located underneath (Figures 2a and 11b–d). The accumulation of bundles of beta-corneous material at the interface between the Oberhautchen and the beta-layer can produce a rich variation in form of micro-ornamentation observed under SEM, from a typical spinulated pattern to the "corneous belt," "dendritic ramification," or the "serpentine-pit and groove" (Figure 12a–c). It is believed that spinulae observed in TEM preparation (Figure 2a) represent the classical spinulated pattern generated from isolated spinulae. However, the present SEM study has revealed that the spinulated surface detected by TEM may also represent areas occupied by "corneous belts" that have been sectioned and appear as spinulae (Figure 12). Also, the dendritic ramification and the "serpentine-pit and groove" pattern, after sectioning, appear as an irregular spinulated pattern. The fusion of few spinulae to form "corneous belts" may derive from an artifactual sticking induced from SEM preparation or from an incomplete detachment of the spinulae tips from the clear layer after shedding (Figure 3b; Alibardi & Bonfitto, 2019, Figure 5b,c). The numerous small or larger fragments of clear layer from the outer epidermal generation still penetrated by the Oberhautchen spinulae (arrowheads in Figure 3b and inset, and Figure 11f) indicate that shedding is not com-pleted over the entire skin surface. However, the fusion of numerous spinulae into long struts of corneous beta-material appears as a true pattern of micro-ornamentation (Figures 3a,c, 8g and 12b).

The complex "corneous dendritic ramification pattern" (Figure 12c) is the more difficult pattern to explain, but similar aspects of the Oberhautchen surface have been observed also in previous SEM studies (Stuart & Daniels, 1972, Figure 5; Peterson, 1984, Figure 4d; Harvey & Gutberlet, 1995, Figure 10a,b; Figure 1b panel right in Griffing et al., 2022; Alibardi & Bonfitto, 2019, Figure 5e). The ramifications represent irregular struts or bundles of beta-corneous material joining Oberhautchen cells with the underlying and compact beta-layer, from which blunted or pointed spinulae emerge in some areas (Figure 12c). Finally, a possible tensile modification of the spinulated surface into a ramification-dendritic shape of the corneous material under stretching conditions, maintained during fixation, may also account for this unusual pattern, as it is often observed in stretched inter-scale epidermis. In fact, the Oberhautchen spinulae may initially be pliable and during scale growth they become harder only after fusion and complete hardening with the beta-layer (Maderson et al., 1998).

**FIGURE 9** SEM images of nuchal (a c) and gular (d f) scales of *L. conraui*. (a) On the outer scale a naked roundish organ (arrowhead) and spinulated sensory organ (arrow) are located. Bar, 50 μm. (b) A small area of the Oberhautchen shows a dendritic pattern among the otherwise spinulated surface. Bar, 5 μm. (c) A circular groove (arrows) apparently separates the spinulated sensillo from the remaining Oberhautchen. Sensitive setae appear to derive from a basal aggregation of corneous struts or roots (arrowheads). Bar, 5 μm. (d) Lateral view of upward oriented scales in the gular region. Bar 50 μm. (e) Dorsal view of gular scales featuring spinulated sensory organs (arrows) positioned in the front part (directed caudally). Bar, 40 μm. The inset (bar, 4 μm) details on the spinulated surface. (f) Gular scale showing the very folded inner surface and the apical sensillo (arrow). Bar, 30 μm. The upper inset (bar, 1 μm) shows the small and blunted spinulae of the inner scale surface. The lower inset (bar, 4 μm) instead shows a transitional area between spinulated and dendritic pattern (arrowheads) present in the inner scale surface. h, hinge region; i, inner scale surface; o, outer scale surface; se, sensillo setae; sp, spinulae

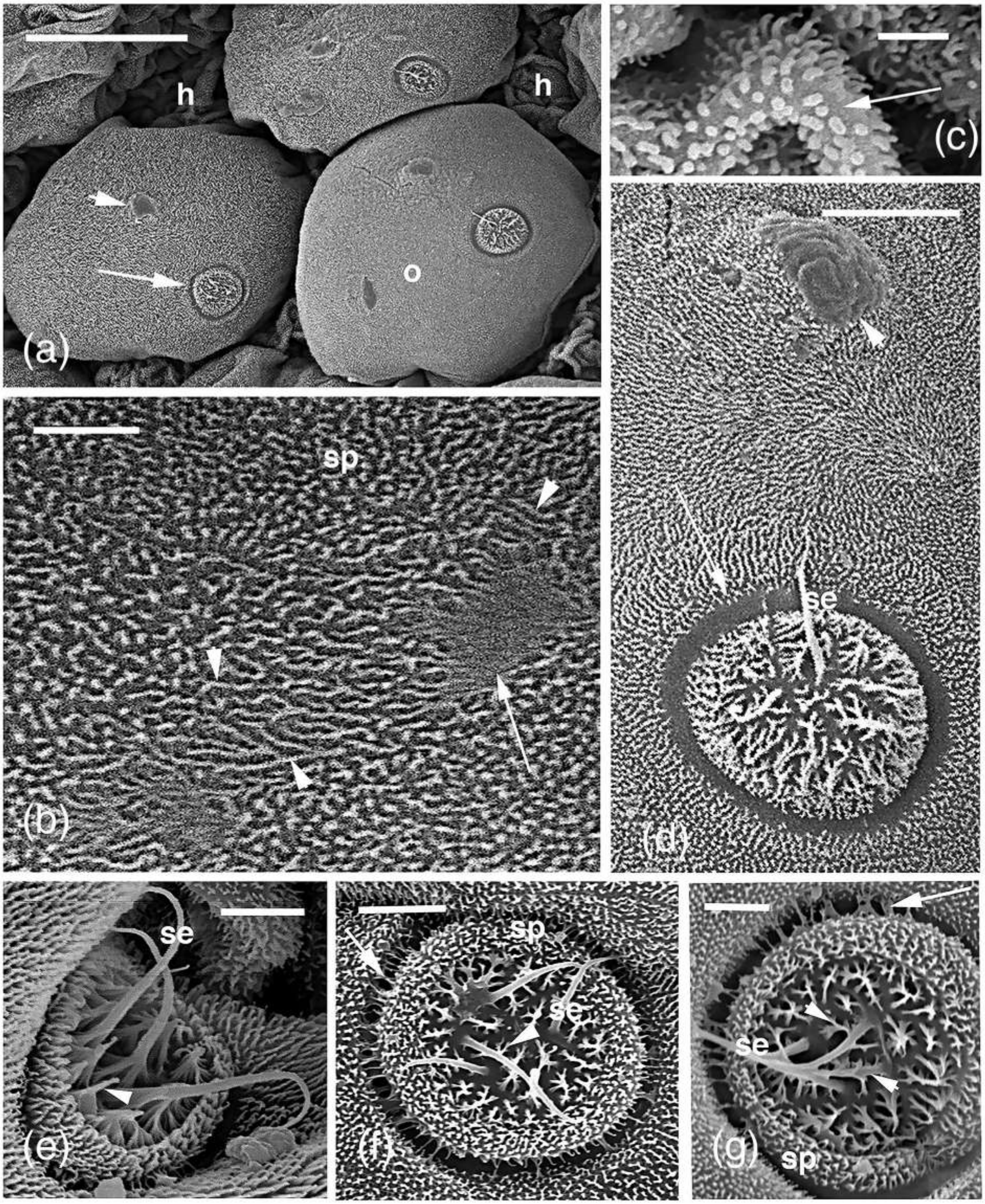

**FIGURE 10** SEM view of fore head scales (a–c) and spinulated sensilli (d–g) of *L. conraui*. (a) Large sensory organ (arrow) and close naked area (arrowhead) on the distal outer side of head scales. Bar, 50 μm. (b) Close up to outer surface area with spinulae, a tissue remnant (arrow) and corneous dendritic figures (arrowheads). Bar, 4 μm. (c) Detail of blunted spinulae present in a fold (arrow) of the hinge region. Bar, 2 μm. (d) Close up view on a spinulated sensillo (arrow) and a lamellar and naked roundish area (arrowhead). Bar, 10 μm. (e) Limb sensillo with long merged spinulae (arrowhead). Bar, 5 μm. (f) Another limb sensillo with peripheral corneous roots (arrow) and central setae with attached roots (arrowheads). Bar, 5 μm. (g) Mid trunk sensillo with a prevalent long seta featuring roots at its base (arrowheads). Numerous corneous roots of the Oberhautchen (arrow) are visible in the outer ring. Bar, 5 μm. h, hinge regions; o, outer scale surface; se, sensorial setae; sp, spinulae

## 4.2 Sensory organ distribution and types

Different types of sensory organs (sensilli) have been described in reptiles (Dujsebayeva, 1995; Ananjeva et al., 1991; Von During & Miller, 1979), and they are generally more numerous in active body regions which movements are very precisely regulated such as the digit extensions connected to adhesive pads contacts and detachment with the substrate (Russell et al., 2021) or on the head (Harvey & Gutberlet, 1995; Jackson, 1977; Lang, 1989; Matveyeva & Ananjeva, 1995; Povel & Van Der Kooij, 1997; Riedel & Schwarzkopf, 2022). The latter study also showed a broad variation in the distribution of sensory organs among species and that the sensilli of terrestrial geckos were smaller but with more bristles than those of the arboreal species (Riedel et al., 2019). In the terrestrial gecko *Teratoscincus scincus* ventral and dorsal sensilli are about the same (Matveyeva & Ananjeva, 1995). The significance of these differences for the ecology of geckos however remains poorly known and mainly speculative.

Three main types of sensilli are described in reptiles, a "spinulated sensory organs with 1–5 single or branched bristles," a flat and "smooth bouton-like tactile organ," and a "knob-like scale organ". Sensory organ with a round shape and covered by flat thin beta- or alpha-layers ("lenticular touch organs") or "knob-like scale organs" or "scale organs," are derived from the local thinning of corneous layers that allow sensory nerves to reach almost the skin surface (Sherbrooke & Nagle, 1996; Von During & Miller, 1979). The naked bumps here noted (Figures 3d, 6g, 8a,d, 9a and 10a,d) resemble sensory areas, like the scale organs described in cordylid and gerrosaur lizards (Harvey & Gutberlet, 1995) or the "spiked scale organs" described in xenosaurid lizards (Harvey, 1993). However typical bouton-like or lenticular "touch sensory organs" were not observed in these species of gecko.

Like other geckos, also in *L. conraui* a slightly higher number of spinulated sensory organs, like those indicates as "setae-bearing sensilla" in the gecko *Tarentola mauritanica* (Hiller, 1977), have been detected by the apex of the tail close to caudal setal pads as well as in the digits (Table 1). In these regions a fine control of tail-digital movements occurs. The low number of spinulated sensory organs present in gular, nape and forehead areas here analyzed indicates that these regions in *Lygodactylus* are less involved in detection and response to mechanical stimuli. A recent study on various Australian geckos however found a lower density of sensilli in limbs scales in comparison to those present in the head and tail tip (Riedel & Schwarzkopf, 2022). These authors indicated that some types of sensory organs may have other sensory capabilities than mechanoreception, such as sensing humidity conditions and local temperatures. Unfortunately, no physiological studies are available on the different sensitivity of otherwise similar spinulated sensory organs among lizards, and only specific experimental studies may explain in future whether these receptors can sense different stimuli.

## 4.3 Hypotheses on the biological roles of micro-ornamentation in *L. conraui*

The numerous studies carried out on micro-ornamentation of lepidosaurians using SEM have suggested several roles for these microstructures in different species related to ecological adaptation and evolutionary diversification (Arnold, 2002; Gower, 2003; Price, 1982; Riedel et al., 2019). The corneous ridges forming micro-ornamentation may extend resistance to abrasion, reduction of sunlight and UV penetration, reflection or iridescence, shining and camouflage, reduction of water permeability, favor locomotion, kill bacteria and protozoans (Arnold, 2002; Gower, 2003; Ruibal, 1968). In general, the reduction of micro-ornamentation and friction appears related to burrowing habits and occupancy of crevices in lizards and snakes. While a smooth surface allows for a better shedding, the increase in minute ornamentation density such as spines enhances shining and iridescence as these microstructures are allocated at regular distances similar or shorter of some light wavelengths, and can disperse/scatter the light into color components. Conversely, increase in micro-ornamentation complexity, also observed in the flat lamellate-spinulated Oberhautchen of numerous snakes is related to improving movement on the substrate (Klein et al., 2010). For two large families of Australian geckos, it was found that terrestrial-adapted geckos possess a higher density and length of spinules than arboreal geckos, likely in relation to efficient self-cleaning and bactericide properties (Riedel et al., 2019).

A strong friction that eventually becomes adhesion is obtained by increasing the length and orientation of micro-ornamentation, in particular in the spiny pattern of anoline lizards and geckos that produces curved spinules of 2–3 μm, then prongs of 4–7 μm, and eventu-ally setae of 15–100 μm (Alibardi, 1999, 2020; Alibardi & Bonfitto, 2019; Bauer, 1998; Griffing et al., 2021; Hiller, 1972; Maderson, 1966, 1970; Peterson & Williams, 1981; Russell et al., 2021). Bizarre micro-ornamentation in some ventral palmar and digit areas observed in spherodactyline geckos of the French Guyane take the shape of spines in some areas,

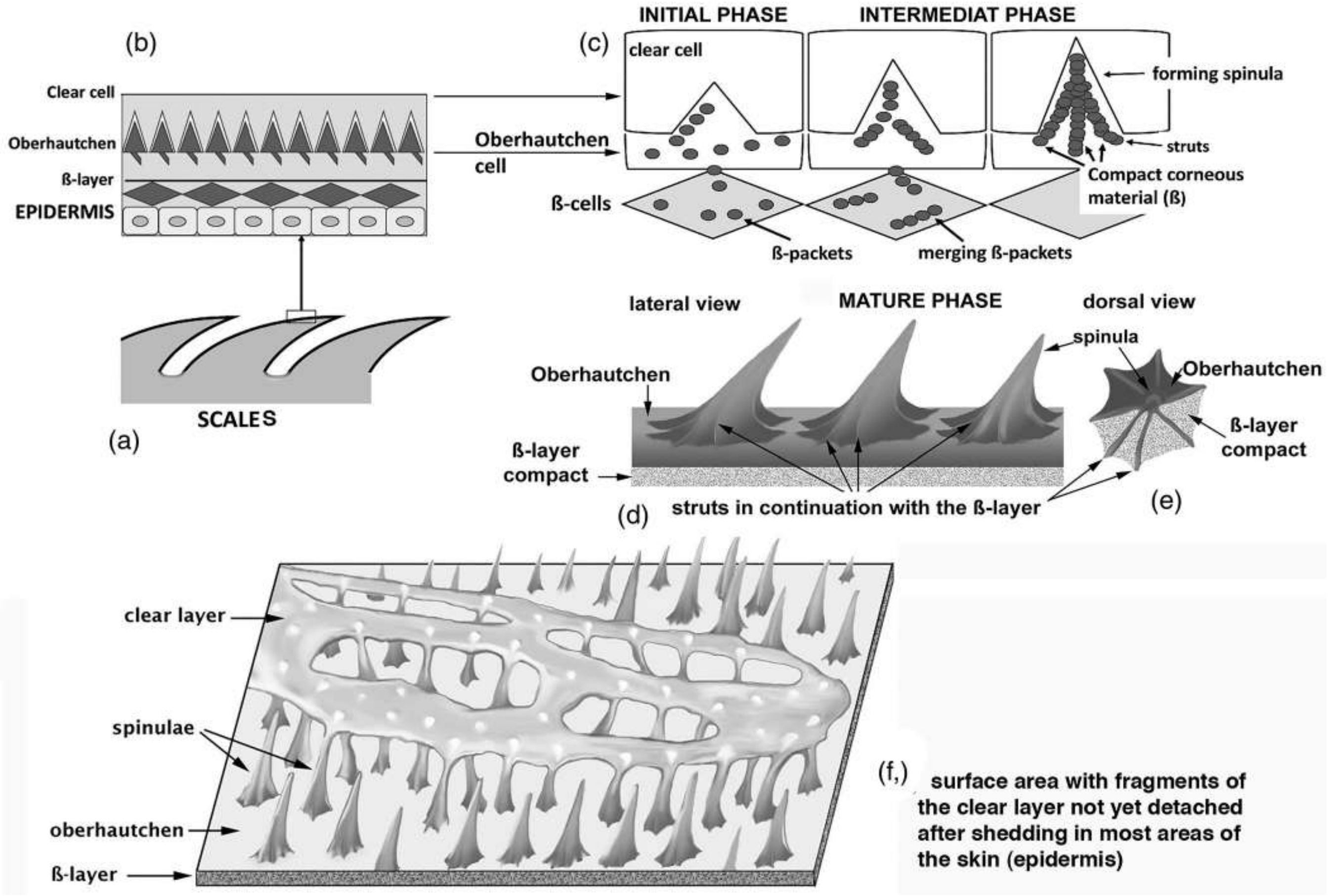

**FIGURE 11** Schematic and self explanatory drawing illustrating the formation of the spinulae in scales (a  c) their mature structure (d, e) and the clear layer fragments present in sparse areas of the epidermis (f). (b) shows a detail of the epidermis of the outer scale surface (a) while c illustrates, from left to right, the progressive formation of spinulae by the accumulation of beta packets containing mainly corneous beta proteins. Spinulae grow inside the above clear layer and later the corneous material becomes compacted into struts (d) that take a pentagonal hexagonal disposition when observed from above (e). (f) Shows a specific area of the epidermis where large clear layer fragments still remain attached to the spines in few areas of the skin

"bouchets" (star-like) in others, and finally setae at the very tip (Gasc et al., 1982; Gasc & Renous, 1980). These different morphologies have been associated with the modality of gait and walking of these small geckos on the fallen leaves layers of the forest where they live.

The above information suggests a functional interpretation of the differences detected among the skin areas of *L. conraui*, an arboreal-adapted and diurnal gecko. From grasping steadily and moving swiftly through the canopy, to lower levels of trees or to vertical walls (Amadi et al., 2017; Manners & Georgen, 2015), some advantages can be useful if the skin surface presents modification in the general spinulated pattern typical of geckos. In the inner scale surface and hinge region the spinulated pattern is very reduced and can transit into a "serpentine or pit-groove pattern" that determines lower friction and facilitate extension and stretching of the skin in these areas. As opposed, the longer spinulae present in the outer scale surface in comparison to the inner and interscale regions determine a higher friction with the substrate, especially in ventral mid-trunk and tail areas (Table 1). Longer spinules (2–4 μm in ventral scales vs. 0.5–1 μm in dorsal scales and in hinge regions) are present in ventral scales of the tail, abdomen, and gular areas where they likely increase friction and attachment to the substrate, and limit slippery on trunks, barks, twigs, and smooth walls. This is also present in the ven-tral scales of the limb that, in addition, present numerous sensory organs that monitor the progression of the gecko movements among the canopy, branches and twigs. After lifting the body over the surface using their legs and, therefore, erasing the frictional effect, the small gecko can swiftly jump from branches to branches. The higher number of sense organs present in the ventral trunk and mid tail in comparison to the corresponding dorsal areas (Table 1) indicates that this part of the body in contact

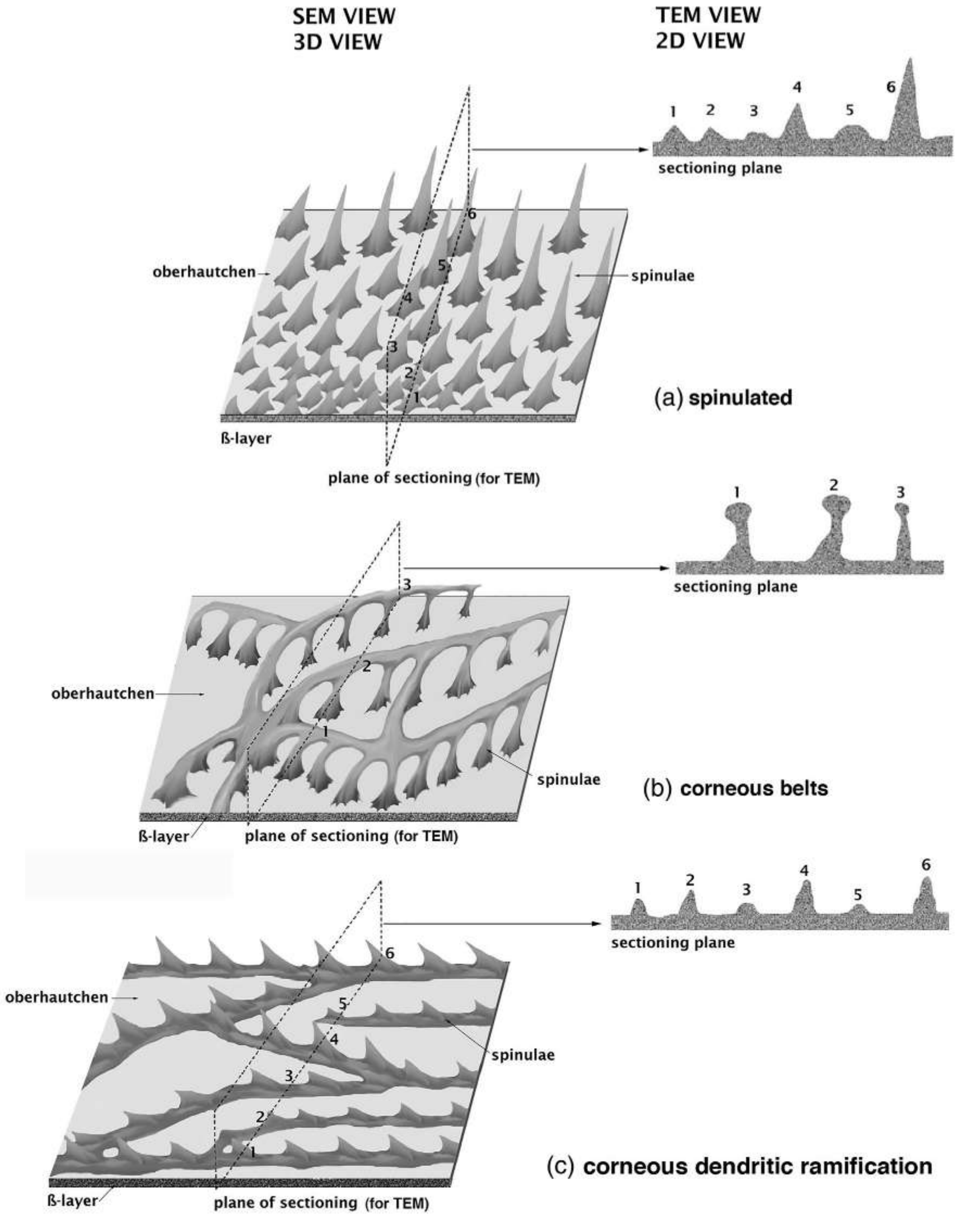

**FIGURE 12** Schematic reconstruction of the three patterns observed in our SEM study (a–c, left) and their different images (profiles) visible under TEM view (a–c, right). The struts are joined to the compact beta layer at the base of spinulae (the smooth grey surface). The drawing shows the different image resulting from a SEM landscape of a "classical spinulated pattern" (a) versus that derived sectioning at random the surface for preparing TEM sections along the sectioning plane. (b, c) are interpretative models of SEM views and their TEM images relative to the "Corneous belts pattern" (b) and the "Corneous dendritic ramification pattern" (c). See text for further explanation.

with different substrates is mainly involved in sensory stimulations. The presence of numerous sensory organs on the ventral side in this arboreal gecko may even allow for detecting vibrational stimuli derived from the movement of insects or their larvae that direct the small geckos to move toward them for feeding, a hypothesis that however remains to be tested by specific studies. This condition is apparently different from that of a terrestrial gecko, *Teratoscincus scincus*, where sensilli are equally distributed in ventral and dorsal scales (Matveyeva & Ananjeva, 1995).

The presence of adhesive pads in the tail tip, aside in the digits, indicates that also the tail is utilized for climbing, a process that requires a high sensory-motor coordination (Bauer, 1998; Griffing et al., 2021; Russell et al., 2021). The small and curved scales of the back have mainly a protective function and are of the tuberculate type, equipped with a relatively thick beta-layer and a lower number of "knob-like" scale organs, and this observation is valid for the nape and head scales that also mainly exert a protective role. The lower number of sensory organs present in these areas indicates that the gecko mainly relays on the sight from his forward and lateral movements among the vegetation. Finally, gular scales contain large inter-scale areas that can be stretched during head movements. This area also in *Anolis carolinensis* presents small scales with extensive inter-scale regions (inner and hinge regions), which in males can be extended into an intensely red gular sac (Alibardi, 2014).

In conclusion, the present study indicates that variations in the spinulated pattern micro-ornamentation and sensory organ distribution present in different body areas of *L. conraui* allow these small geckos an optimal friction, adhesion, movement coordination, and monitoring vibrational stimuli coming from the arboreal environment where they live.

## AUTHOR CONTRIBUTIONS

**Lorenzo Alibardi:** Conceptualization (lead); data curation (equal); formal analysis (equal); funding acquisition (supporting); investigation (supporting); methodology (supporting); project administration (equal); resources (supporting); software (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (lead); writing – review and editing (lead). **Davide Bussinello:** Conceptualization (supporting); data curation (supporting); formal analysis (supporting); funding acquisition (supporting); investigation (supporting); methodology (supporting); project administration (supporting); resources (supporting); software (supporting); supervision (supporting); validation (supporting); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Antonio Bonfitto:** Conceptualization (supporting); data curation (equal); formal analysis (lead); funding acquisition (lead); investigation (equal); methodology (lead); project administration (equal); resources (lead); software (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (supporting); writing – review and editing (supporting).

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

There are no other data other than those reported in the article.

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