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Comparative Study of the Pheromone-manufacturing Femoral Glands in Two Sympatric Species of Lacertid Lizards (*Acanthodactylus*)

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Femoral glands are holocrine structures that produce compounds used by lizards as pheromones. Few studies have investigated the morphology and ultrastructure of these glands. We chose a closely related species pair from a lizard family having femoral glands in male and female of both species to illustrate comparative morphology and ultrastructure and their implications for the mechanism of secretion dispersal to the environment. We also aimed to test whether the structure and mechanism of secretion production differ between related species. In addition, we sought to gain a better understanding of the holocrine mechanism of secretion. Femoral glands of selected sympatric lacertid species, *Acanthodactylus boskianus* and *A. scutellatus* were studied comparatively using light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). SEM revealed both interspecific and sexual variation in the morphology of the glandular pores. The external morphology suggests the mechanism of the secretion deposition where the convex part of pore-carrying scale is probably used to partition the secretory plug. Histology shows the epithelial cells of the gland duct as an extension of the epidermis with its covering keratin. The glandular acini are composed of germinal and secretory cells. The latter undergo four different stages of differentiation, from the beginning of the formation of secretory granules, through the accumulation of these granules, disintegration and formation of the secretory plug, which protrudes externally. The study considers the sequence of holocrine secretion development, and explains in part how such secretions are deposited on the substrate. Sexual differences at the external morphology level were more evident than interspecific differences.

Key words: *Acanthodactylus boskianus*, *Acanthodactylus scutellatus*, femoral glands, pheromones, lizard behaviour, exocrine glands

INTRODUCTION

Reptilian skin is usually described as dry and largely devoid of glands. Generally, there are two types of epidermal glandular specializations in the lizard epidermis (Maderson, 1967, 1968). The first are generation glands, which are represented by patches of scales on the forelimb, posterior abdominal, or femoral regions of the lizard body (Louw et al., 2010). These are holocrine epidermal glands, which start to differentiate with the onset of sexual maturity (Mouton et al., 2010) and are composed of several layers of mature glandular material. The second type are femoral and precloacal (pre-anal) glands. Histologically, the femoral or precloacal glands may be follicular or tubular and open to the exterior by a well-defined pore (Cole, 1966a). During the breeding season, the stratum germinativum actively produces secretory cells that go through a series of stages culminating in the release of secretory materials to the external environment (Cooper et al., 1999; Khannoon, 2004).

Otth (1833) was among the first authors to discuss the precloacal and femoral glands of lizards, and Schaefer (1902) and Abraham (1930), among others, provided detailed histological descriptions of these structures. Cole (1966b) provided a histological description of the femoral glands in the crotaphytid iguanian *Crotaphytus collaris*. He demonstrated that these glands are composed of branching tubes and tubules. Precloacal glands of two gekkonid lizards, *Gekko gecko* and *Hemidactylus bowringii* were described by Chiu and Maderson (1975). The glands of the former were tubulo-acinar, whereas in the latter the glands were simple tubular. Subsequently, more studies described these glands histologically. Chauhan (1986) indicated that the pre-cloacal glands of *H. flaviviridis* are tubulo-acinar. Khannoon (2004) histologically studied the glands of five different lizards, two agamids, two lacertids and one gekkonid. These were of two types: tubulo-acinar in lacertids and gekkonids, and branched tubular in agamids. More recently, the femoral glands of a variety of other lizards have been the object of study (Imparato et al., 2007; Louw et al., 2007, 2011; Labra, 2011; Chamut et al., 2009; Mouton et al., 2010).

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Integumental gland secretions in lizards play a role as semiochemicals (Maderson, 1985, 1986; Jared et al., 1999; Aguillar et al., 2009; Khannoon, 2009; Khannoon et al., 2010, 2011b). Working on iguanids, Alberts (1989, 1991, 1992, 1993; Alberts and Werner, 1993; Alberts et al., 1993) indicated that both field and laboratory studies suggest that femoral gland secretions function in conspecific recognition and range marking. The secretions consist of both lipids and proteins (Mason and Gutzke, 1990; Alberts, 1990) and the major compounds involved in chemical communication are thought to be lipids (Lemaster and Mason, 2001), although Alberts et al. (1993) recorded important differences in femoral gland proteins between populations, kin groups, sexes, and individuals.

Acanthodactylus boskianus and *A. scutellatus* are medium to large sized sympatric lacertid lizards, inhabiting deserts and semi-deserts in North Africa and the Middle East. As in other lacertids, these species have single row of epidermal glands on both femurs in both males and females. These glands secrete semisolid secretory plugs. The secretory activity of these glands varies seasonally, decreasing before and during brumation and increasing during the reproductive season (personal observation for the secretory plug development). The femoral gland secretions of *A. boskianus*, contain semi-volatile chemicals that elicit sex-specific responses in conspecifics (Khannoon et al., 2010, 2011b). These glandular secretions, which are passively deposited on the ground during movement, or actively when the hind legs are dragged, have a characteristic compound bouquet typical for *A. boskianus* (Khannoon et al., 2011a). *Acanthodactylus scutellatus* is a closely related species and live in sympatry with *A. boskianus*. The chemical composition of femoral gland secretions in *A. scutellatus* is unknown but, in light of the behavioral context and chemical identification of glandular secretions in *A. boskianus*, we undertook a morphological and ultrastructural evaluation of the glands in both species in order to characterise the morphological and anatomical properties of *Acanthodactylus* femoral glands across species and sexes.

MATERIALS AND METHODS

Samples

Adult lizards used in the present study were collected from Balteem, Egypt (31°33′ N, 31°05′ E) and were identified according to recent reviews of Egyptian reptiles (Saleh, 1997; Baha El Din, 2006). Five animals of each sex of both *Acanthodactylus boskianus* and *A. scutellatus* were used for the histological and ultrastructural study. A Zeiss stereo microscope was used for checking and counting the femoral pores of 68 males and 67 females of *A. boskianus* and 19 males and 5 females of *A. scutellatus.*

Histology

Skin and underlying tissue were removed from the femoral region of *Acanthodactylus* spp. Small pieces were directly fixed in Bouin's fluid or neutral buffered formalin solution, then washed and dehydrated in ascending grades of alcohol, cleared in xylene and embedded in two or three changes of paraffin wax (m.p. 56–58°C). Paraffin sections were cut serially at 5–7 μm thickness. Slides were stained with hematoxylin and eosin using standard methods (Humason, 1979) to investigate the epidermal glands.

Ultrastructure

Pieces of the gland were processed for electron microscopy, fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer (PH 7.2), after fixation, specimens were washed in 0.1 M cacodylate buffer (pH 7.2) and post-fixed in 1.0% osmium tetroxide for 2 h. Specimens were then dehydrated in a graded ethanol series, transferred to propylene oxide and embedded in Embed 812 epoxy resin. Semi-thin sections (1 micrometer) were cut with glass knives on an RMC 6000 ultramicrotome and were stained with 1% toluidine blue. Ultra-thin sections (approximately 80 nm) were stained with uranyl acetate and lead citrate. Examination was performed in a Hitachi H-7600 transmission electron microscope operating at 80 kV and photographed with an AMT XR 40 bottom mount digital camera.

Scanning electron microscopy

Pieces of skin from the femoral region containing the femoral pores were removed and fixed in 10% buffered neutral formalin, rinsed in cacodylate buffer, and post-fixed with osmium prior to dehydration in an alcohol series. Specimens were critical point-dried in a Polaron E3100 critical point drier using liquid carbon dioxide. Samples were mounted on stubs and coated with gold/palladium in a Polaron SC 7640 sputter coater and examined on a Hitachi S570 Scanning Electron Microscope operating at 5.0 kV, and imaged using EDAX Genesis software and hardware.

RESULTS

External morphology

In both *A. boskianus* and *A. scutellatus* males and females have femoral glands. The external pores of the glands are located on the ventral side of each thigh. In *A. boskianus* the male exhibit 16–29 pores and the female exhibit 18–29 pores on each thigh. However, males with a low number of pores were rare. In *A. scutellatus*, the male has 22–29 pores and the female has 22–26 pores on each thigh. In *A. boskianus*, males had a significantly higher number of total femoral pores (48.57 ± 0.68) than females (45.40 ± 0.78), (F = 2.30, P = 0.003). In *A. scutellatus*, there was no significant difference in the total femoral pores between males (48.63 ± 0.63) and females (48.50 ± 1.26), (F = 0.143, P = 0.93).

Scanning electron microscopy

The secretory plug protrudes from the gland pore above the skin surface. There are sexual but not interspecific differences in the external morphology of the gland revealed by SEM. The pore and consequently the secretory plug are flattened, laterally compressed, about three times longer than wide, and their long axis is perpendicular to the axis of the thigh in males of both *A. boskianus* and *A. scutellatus* (Fig. 1A–C). In females of both species, it is in the form of a rod with a round contour and circular pore (Fig. 1D–G). The plug in all cases is completely enveloped by a smooth sheath. Higher magnification of the plug content shows the dead cells as entire entities in contact, and the secretory granules can be discriminated. The plug emerges from a pore-bearing scale in both sexes of both species. This scale has two lips. The dorsal lip is the larger (about 580 μm at maximum width) and is convex and the ventral lip is the smaller (about 280 μm at maximum width) dome-shape in appearance (Fig. 1).

Histology

Generally, males and females have well-developed glands (Fig. 2F, G). In histological section (Fig. 2), the structure of the femoral glands was similar between the two species and between sexes. The glands (Fig. 2A) are tubuloacinar in form. The secretory duct (Fig. 2B) of the gland runs almost vertically, from the secretory pore through the deep

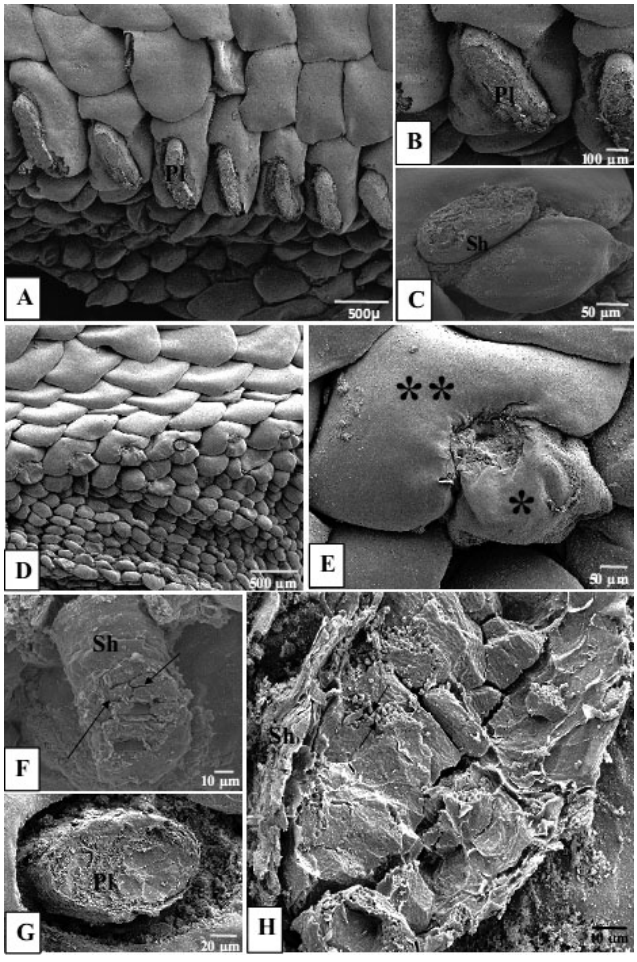

Fig. 1. Scanning electron micrographs: **(A)** Successive femoral pores of male *A. boskianus*. **(B)** One pore with flattened opening and exhibiting a flattened plug (Pl). **(C)** The plug in male *A. scutellatus* enveloped by a smooth sheath (Sh). **(D)** Successive rounded femoral pores of female *A. boskianus*. **(E)** The pore-bearing scale of female *A. boskianus*, note the two lips; convex dorsal (**) and dome-shape ventral lips (*). **(F)** and, **(G)** Secretory plug of female *A. scutellatus* showing the smooth sheath covering the plug and the desquamated cell entities (arrows) in the plug. **(H)** Magnified plug showing the sheath and the secretory granules (arrows) in the plug.

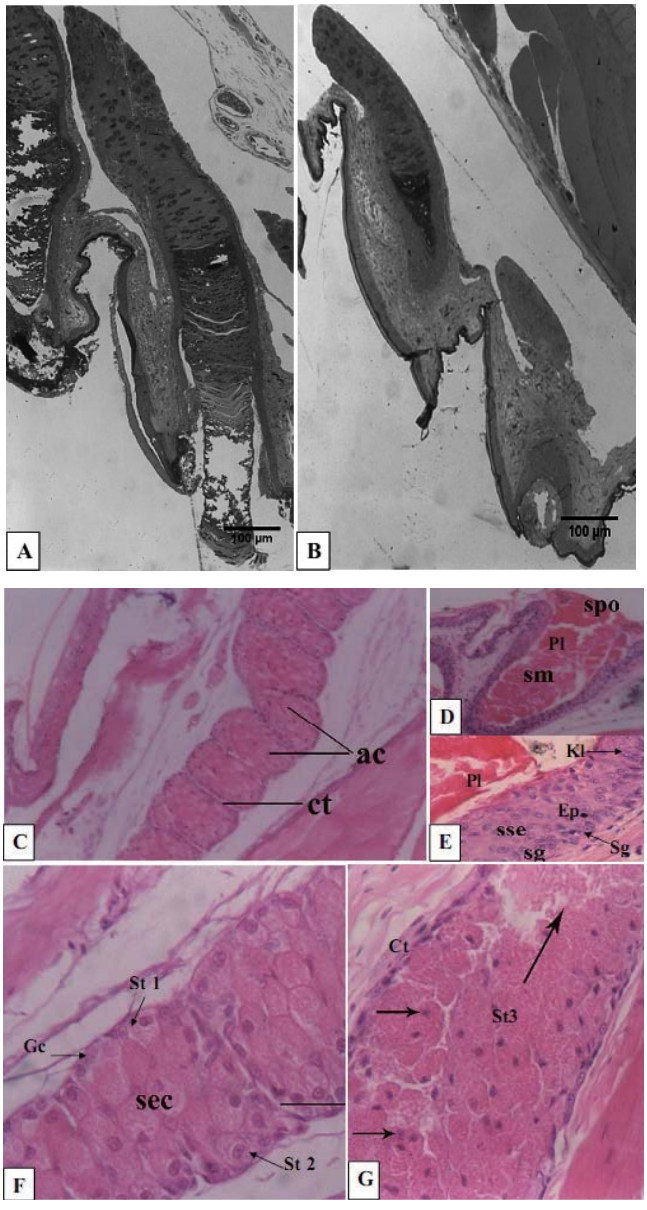

Fig. 2. Light micrographs showing the histology of femoral glands of *Acanthodactylus* lizards, stained with hematoxylin and eosin. **(A)** and **(B)** Histological sections through the whole gland of both male and female *A. boskianus* respectively, showing that the glands are well developed in both sexes. **(C)** Section through the gland of male *A. boskianus* showing the acini (Ac) separated by connective tissue (Ct), Magnification: 400×. **(D)** Secretory pore of female *A. boskianus* showing the extension of epidemis into the duct of the gland; plug (Pl), secretory matter (sm), secretory pore (Spo), 100×. **(E)** Duct wall of the gland showing stratified squamous epithelium (sse), keratin layer (Kl), epidermis (Ep), stratum germinativum (Sg), 800×. **(F)** Different stages of secretory cells magnified from (C); germinative cells (Gc), secretory cells (Sec), First stage (St1), second stage (St2), 1000×. **(G)** Third stage (St3) of secretory cells forming the secretory plug; connective tissue (Ct), pycnotic nuclei (small arrows), direction of the secretions movement towards the pore (large arrow).1000×.

dermal tissues of the skin, and then runs anteriorly beneath the deep dermal layer. The secretory duct is lined by thick stratified squamous epithelium, 3–7 cell layers thick, the germinative layer of which is continuous with stratum germinativum of the skin (Fig. 2C). The proximal third consists of closely-juxtaposed interwoven acini separated by connective tissue septa, while in the middle third the acini join to form a longitudinally oriented duct. The distal third is represented by a single ovoid secretory duct leading to the secretory pore. The acini (Fig. 2D) consist of germinal cells and secretory cells. The non-differentiated peripheral germinal cells have basophilic cytoplasm. These are flattened or cuboidal cells with ovoid nuclei. The central secretory cells of the acini are larger and represent most of the acinar volume. These are more eosinophilic than germinal cells with rounded or ovoid nuclei. Using haematoxylin and eosin staining, only three secretory cell stages were observed. Cells in the first stage appear with central nuclei and eosinophilic cytoplasm (Fig. 2D). Second stage cells have granular cytoplasm due to the presence of secretory material. At the

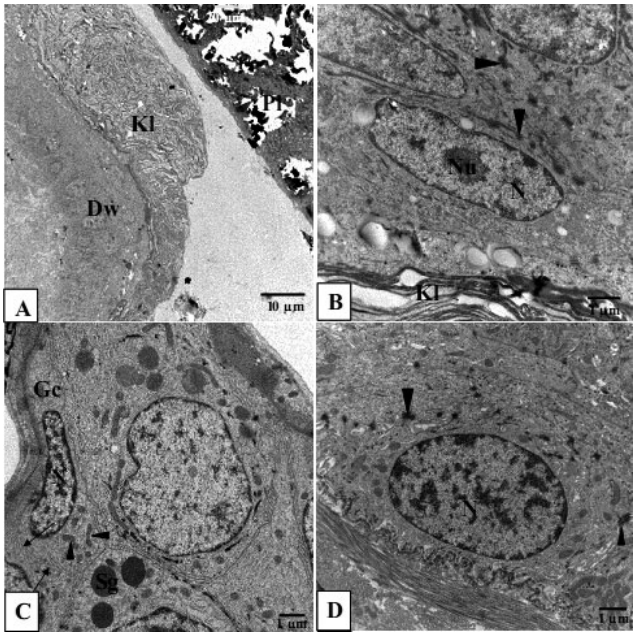

Fig. 3. Transmission electron micrographs of the duct wall and germinal cells of the femoral glands: **(A)** The gland duct wall (Dw) around the secretory plug (Pl), the duct wall consists of epithelial cells covered by keratin layers (Kl). **(B)** Epithelial cells of the duct wall with ovoid nuclei (N) and prominent nucleolus (Nu). Many desmosomes (arrowheads) are found linking these cells. **(C)** Germinal cells (Gc) at the periphery of the acini with ovoid or flattened nucleus, numerous organells like mitochondria (arrowhead) are observed. Interdigitations (arrows) between the germinal cells and secretory cells, the latter having secretory granules (Sg), are obvious. **(D)** Desmosomes (arrowheads) link the germinal cells with the neighbouring secretory cells.

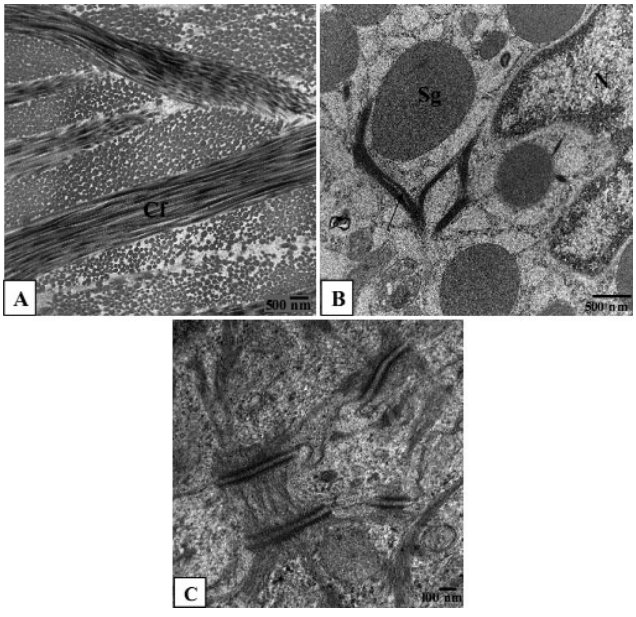

Fig. 4. Transmission electron micrographs showing structures and organelles from secretory cells ofstage 1: **(A)** Collagen fibers surrounding the gland. **(B)** Bundles of tonofilaments (arrows) are common features of these cells. **(C)** Desmosomes are magnified to exhibit basic structure.

distal end of the gland (Fig. 2E), the cells of the third stage, which are filled with secretory granules, are located close to the pore, the nuclei appear pycnotic and nuclear fragmentation increases and, finally these form the secretory plug. The secretory plug, which protrudes externally through the pore of the gland stains a very light pink with eosin and fragmented during sectioning.

Ultrastructure

Similar structure was observed in both sexes. At the periphery of the acini, the germinative cells are small and polygonal or spindle-shaped (Fig. 3C). The nuclei, which occupy the majority of the cell volume, are likewise spindle-shape or polygonal, and are sometimes lobed. Loose chromatin and nucleoli are a common feature of these cells. Numerous organelles could be seen; smooth and rough endoplasmic reticulum, mitochondria, and Golgi apparatus.

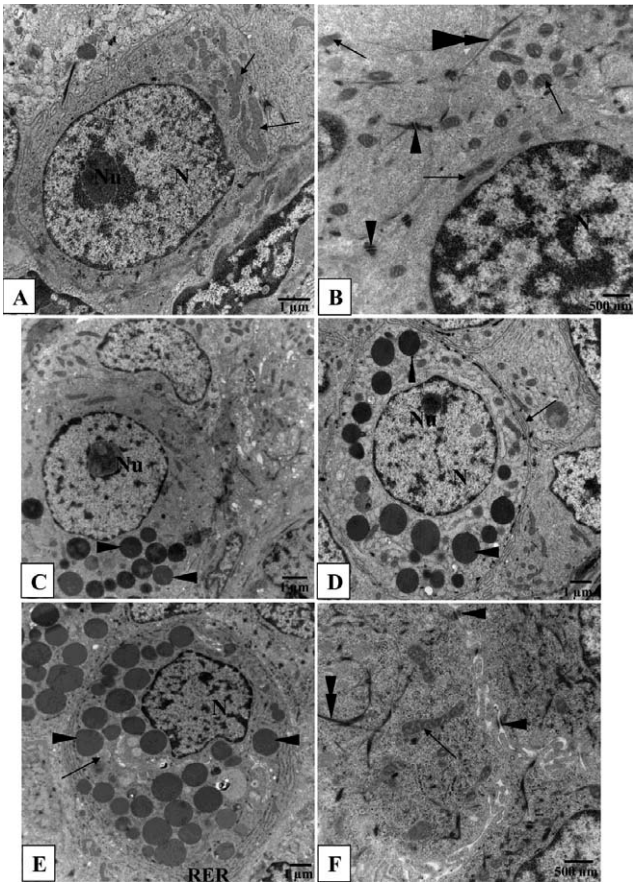

Fig. 5. Transmission electron micrographs showing secretory cells at stages 1 and 2: **(A)** and **(B)** Stage 1, cells with voluminous nuclei and obvious nucleolus, interdigitations, tonofilaments (double arrowheads) and desmosomes (arrowheads) as well as organelles like mitochondria (arrows) are observed. **(C–F)** Stage 2, with variable numbers of secretory granules (arrowheads). **(D)** Interdigitations (arrows) are common. **(E)** Lipid droplets (arrows) are observed among secretory granules, and rough endoplasmic reticula (RER) are frequently observed. **(F)** Desmosomes (arrowheads) and interdigitations (between the two arrowheads) are common characters, tonofilaments (double arrowheads) and organelles, such as mitochondria (arrows), are common.

These cells are characterized by many desmosomes, and tonofilaments can be seen linked to desmosomes or inside the cells (Fig. 3D, Fig. 4A, B). Collagen fibers surround the gland acini (Fig. 4C).

The secretory cells are characterized by the presence of secretory granules, the density of these granules depends on the cell stage. Although there is a continuum of variation across the secretory cells, TEM enabled us to observe more stages other than those appeared using light microscopy. Four stages of secretory cells have been recorded to simplify their description. In the first stage (Fig. 5A, B), the secretory cells are approximately spherical or ovoid with spherical nuclei, and are larger than the germinative cells. Mitochondria and granular endoplasmic reticulum are more frequently observed than in other stages. No secretory granules are present at this stage. Desmosomes and interdigitations are numerous. In the second stage (Fig. 5C–F), secretory granules are present. Ovoid or circular nuclei are clearly visible. Granular endoplasmic reticulum is evident.

At stage three (Fig. 6A–D), the secretory granules increase and push the nucleus, which becomes more electron dense, and lobed or irregular in shape, towards the cell periphery. The nuclei are less regular in shape and at late stage three they can show invaginations due to the pressure of the secretory granules. Nearly all the secretory granules are homogenous, but differ in electron density. Lipid droplets, scattered in the cytoplasm, are clearly visible at this stage. At late stage three, the secretory cells become almost fully occupied by secretory granules. Unknown dense polygonal or spindle-shape bodies, resembling secretory material, were recorded between cells (Fig. 6E). Granular endoplasmic reticulum could be seen at the periphery of the cells.

In the fourth stage (Fig. 7), the cells are elongated or spindle-shaped, and are arranged perpendicular to the longitudinal axis of the glandular duct. The secretory granules fully occupy the cytoplasm and no organelles can be seen. Nuclei, if present, are pycnotic at the cell periphery. These dead cells are compressed towards the glandular duct forming a secretory plug.

The layers of the duct of the gland, which are continuous with the skin epidermal layers show the characters of epithelial tissue (Fig. 3A). These cells are flattened or polygonal and lie above the stratum germinativum (Fig. 3B). The latter is represented by cuboidal cells with voluminous rounded nuclei. The epithelial cells have ovoid or flattened nuclei, and frequent organelles, like mitochondria, are observed. Extensive interdigitations and a high number of desmosomes are seen between these cells.

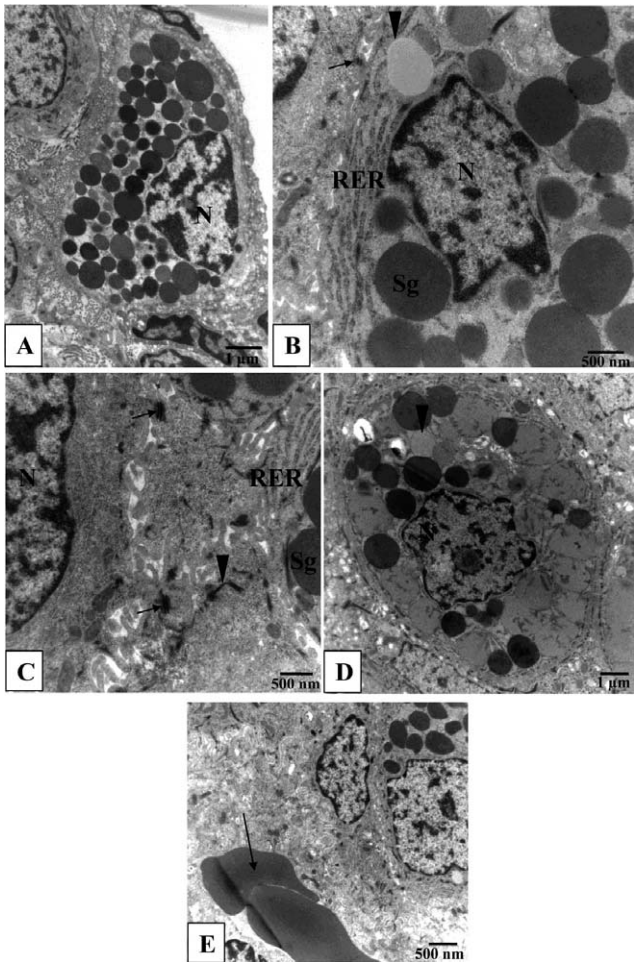

Fig. 6. Transmission electron micrographs showing secretory cells at Stage 3: **(A)** Secretory cell are filled with secretory granules. **(B)** Viable organelles such as RER are still observed, lipid droplets (arrowhead) and secretory granules (Sg) occupy the majority of the cell; desmosomes (arrows) are still observed. **(C)** Desmosomes (arrows) and tonofilaments (arrowheads) and some organelles are characteristic of these cells. **(D)** More lipids droplets (arrowheads) are common. **(E)** Unknown secretory-like large body (arrow) is observed between cells.

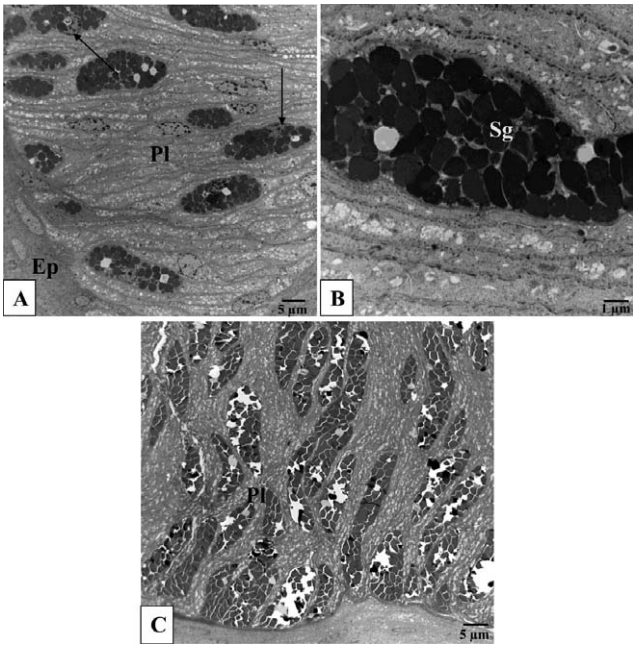

Fig. 7. Transmission electron micrographs showing secretory cells at Stage 4: **(A)** and **(B)** The cells are filled with secretory granules and no viable organelles can be observed; pycnotic nuclei (arrows) are frequent, these cells constitute the secretory plug (Pl) enveloped by the epidermis (Ep) of the gland duct. **(C)** Secretory plug consists of desquamating secretory cells, the plug is artificially fractured.

DISCUSSION

Our histology and ultrastructure results showed no interspecific or intersexual differences in femoral secretory glands in these species. On the other hand, external morphology showed sexual dimorphism. The glands in both *Acanthodactylus boskianus* and *A. scutellatus* open through femoral pores that lie in a linear series on the ventral aspect of each thigh, similar to other lizards (Cole, 1966b; Menchel and Maderson, 1975; Chauhan, 1986; Imparato et al., 2007). On the other hand, some geckos (Chauhan, 1986; Chiu and Maderson, 1975; Khannoon, 2004) and amphisbaenians (Jared et al., 1999) have these glands restricted to the area immediately anterior to the cloaca.

In most lizard families these pores are well developed in males, but less pronounced or absent in females. The well-developed pore and subsequently well-developed gland play a role in territorial marking, an activity that, in most lizards, is usually restricted to males (Alberts, 1991; Alberts et al., 1993; Martin and Lopez, 2007; Khannoon, 2009; Khannoon et al., 2011b). In our study, the pores are well developed in females. The role of female glandular secretions remains poorly understood, but the presence of femoral glands and pores in males of some non-territorial lizards, such as teiids and gymnophthalmids, suggests that pore presence need not imply territoriality. Also, symmetry in the number of femoral pores is an important criterion in the male dominance in lacertid lizards (Martin and Lopez, 2007). They have shown that dominant males tend to have greater symmetry.

The pore-bearing scale is divided into two pieces; a large convex piece and a small dome-shaped piece. This is different from the situation in other lizards studied. The teiid lizard *Ameiva ameiva* has rosette-like pore-bearing scales (Imparato et al., 2007), and a similar morphology occurs in other lizards, including iguanids and even in some lacertids (Cole, 1966a; Blasco, 1975). We suggest that the convex part of the pore-bearing scale may be useful in "cutting" the secretory plug, particularly if this happens actively during dragging of the hind limb. In addition, we agree with Imparato et al. (2007) that the pore-carrier differentiated scales derive from the fragmentation of a single scale.

The femoral glands of both *Acanthodactylus* species examined in this study are tubulo-acinar structures. Similar gland construction has been recorded in the geckos *Gekko gecko* (Chiu and Maderson, 1975), and *Hemidactylus falviviridis* (Chauhan, 1986). The femoral glands of *Crotaphytus collaris* (Cole, 1966b) and *Amphisbaenia alba* (Antoniazzi et al., 1994; Jared et al., 1999) have a branched tubular structure. On the other hand, femoral glands in the gecko *Hemidactylus bowringii* (Chiu and Maderson, 1975) and in the agamid *Uromastyx hardwickii* (Athavale et al., 1977) have tubular glands. The epidermal glands are embedded in the dermis of the scales anterior to their pores and are connected to them by elongated ducts. This position is similar to that of previously studied teiids, agamids, gekkonids, cordylids, and lacertids (Blasco, 1975; Chiu and Maderson, 1975; Chauhan, 1986; Imparato et al., 2007), but differs from that in the crotaphytid iguanian *Crotaphytus collaris* (Cole, 1966b) in which the glands are situated immediately deep to the pores.

Germinal cells, compared to secretory cells, have the same epidermal characters with voluminous nuclei, many desmosomes, interdigitations, and tonofilaments. Under transmission electron microscopy, it was possible to distinguish four stages of secretory cell differentiation. These stages of cell differentiation resemble what has been observed in the epidermal glands of amphisbaenians (Antoniazzi et al., 1993, 1994; Jared et al., 1999) and in other lizards (Cole, 1966b; Maderson, 1972; Imparato et al., 2007), although the number of secretory cell stages is somewhat arbitrary, and different authors have subdivided the process into a variable number of stages. In the present study, the secretory cells, particularly in late stages, show lipids vesicles which constitute the main part of the secretory matter structure characteristic of this species (Khannoon et al., 2011a).

Scanning electron microscopy identified sexual variation. Males of both species studied have similar flattened secretory plugs and pores, while females showed a rod-shape plug and circular pore. However, in both species, the plug is enveloped by a sheath similar to that described by Cole (1966b) as a keratin layer-covered surface of the female gland. This sheath, in addition to maintaining the cohesion of the secretions in the form of in-contact pieces, ensures that the plug erodes in relatively large pieces, rather than one or a few cells at a time. The plug continues to protrude over the skin surface and then separates into pieces passively or actively by dragging the hind legs (personal observation), particularly in males. The position of such ventral pores facilitates passive secretion deposition in the territory. However, adult male *A. boskianus* have been observed to actively drag their legs over rocks to ensure secretory deposition (Khannoon et al., 2010, 2011b).

According to Alberts (1993), the distal portions of lizard femoral gland secretion plugs are fractured and deposited on the substrate, where they remain in the form of secretion blocks. This is the same situation described for the teiid lizard *Ameiva ameiva* (Imparato et al., 2007) and proposed for *Acanthodactylus* in this study. Passively or actively, the lizards leave traces of these secretions in the environment and we assume this is facilitated by the described position of the pores and secretion plugs. The secretions, which are semi-volatile chemicals (Khannoon et al., 2011a) deposited in a desert environment, must remain on the substrate for enough time to convey their chemical message in the territory (Khannoon et al., 2010, 2011b). Such pieces have a relatively small surface area to volume ratio and thus low molecular weight compounds will be released slowly from the plug pieces as they degrade, rather than rapidly evaporating as would occur if the secretions were abraded.

A different mechanism of deposition in amphisbaenians was demonstrated experimentally by Jared et al. (1999) using SEM and secretion trailing. As a result of the position of these glands, the secretion plugs, perpendicular to the body, make direct contact with the tunnel walls where these reptiles live (Antoniazzi et al., 1993, 1994).

Desmosomes are adhesion sites that are associated with the cytoplasmic intermediate filament cytoskeleton providing mechanical stability in epithelial cells, thus connecting adjacent cells and forming mechanical support of the tissue. Despite being in different secretory stages, the secretory

cells still have desmosomes and interdigitations. We think that one of the reasons why the plug is produced as one piece and the secretory material is adhesive is the presence of such desmosomes. This is an important factor in keeping the secretory material in contact to promote the persistence of the secretion odor through the deposition of relatively bigger pieces of the secretory plug. In *Amphisbaena alba* (Amphisbaenidae) (Jared et al., 1999), the dead polyhedral cells composing the plug, in contrast, present a discontinuous composition, each cell being broken into small fragments. In this amphisbaenian, the secretion granules do not completely coalesce and individually constitute the small fragments of the plug, finally constituting what is called pheromones-sachets proposed by (Antoniazzi et al., 1993). This is functionally significant in *Amphisbaena alba*, which makes direct contact with the tunnel walls in which it burrows (Antoniazzi et al., 1993, 1994), in contrast to the xeric-adapted lacertids studied here.

Jared et al. (1999) described a different type of cell within the amphisbaenid gland, so called "embracer cells" which are typical elongated and flattened keratinocytes, involving each one of the polyhedral cells. They suggested that these embracer cells may play a role in supporting the polyhedral secretory cells in the gland. The polyhedral cells and embracer cells were forming a functional unit constituting the trail on the ground. This type of cell might be unique to amphisbaenians; it was not detected in *Acanthodactylus* spp., nor has it been reported in other lizards.

We can summarize the role of these glands by the mechanism of secretion. The germinal cells produce secretory cells that sequentially fill with secretions, gradually increase in number and size of secretory granules, and eventually die. These dead cells (containing concentrated secretions) are pushed out through the main gland duct at the distal end of the gland, while more secretory cells are added at the proximal part of the gland. In this regard femoral pores function much like the sebaceous glands of mammals (Bell, 1974; Jenkinson et al., 1985). The secretions are represented by a secretory plug that exudes through a secretory pore. Accumulation of secretions pushes the plug above the skin surface of the ventral surface of the thigh. During movement of the lizard, the secretions will be separated, particularly if the plug is sufficiently extruded from the pore, and this may be assisted by the morphology of the pore-bearing scale, as discussed above. Otherwise, the lizard may actively touch or drag the hind limb over the ground or specific rocks leaving the secretion trails. The secretion trails will be assessed by conspecifics by tongue flicking (Khannoon et al., 2010, 2011b), at least for the lipid fraction of the secretions. The biological role of the apparently similar secretions of *Acanthodactylus scutellatus* has not been studied, but is presumably similar. It is shown from the present study that the femoral glands show sexual variability in external morphology in addition to the chemical differences recorded previously.

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