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# Functional Morphology of Femoral Glands in the Tegu Lizard, *Tupinambis merianae*

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**Several lizards have femoral glands, which have an influence in various reproductive behaviors. In this paper we describe the morphological organization of the femoral glands in the Tegu, *Tupinambis merianae*, by means of light and electron microscopy. Even though these glands are present in both genders, secretions during the reproductive period can only be found in males. The glandular parenchyma, which is organized in numerous secretory units, consists of keratinocyte-like cells and granular cells. The holocrine secretion is constituted from both cells, which lose their integrity and become a semi-amorphous material, reinforced by keratin sheets. The discharges of each unit merge together into a solid cylinder of secretion, surrounded by epithelial cells, that is extruded to the exterior. The keratin sheets and epithelial layers that surround both the complete and partial secretions form a sort of structural support suitable for the type of territorial demarcation characteristic of the species. The granular cells, supposedly the producers of pheromones, are characterized by the presence of electron-dense granules and multilaminar membranous bodies that show ultrastructural changes of unknown function. The free granules in the secretion cylinder may act as pheromone deposits.**

**Key words:** *Tupinambis merianae*, Tegu lizard, femoral glands, morphology, territorial demarcation

## INTRODUCTION

Chemoreception seems to play a key role in the social behavior of reptiles (Halpern, 1992; Mason, 1992). Numerous facts show the influence of pheromones in the sexual conduct of lizards (Cooper et al., 1986; Cooper and Vitt, 1986; Cooper and Garskta, 1987) however, the structural aspects of the organs involved in the production of these substances are poorly known, especially in Neotropical lizards (Jared et al., 1999; Imparato et al., 2007). Even though several of the glandular elements in the skin of squamate reptiles have been described (Maderson, 1967; van Wyk et al., 1992), femoral glands seem to be the most common semiochemical source (Chauhan, 1986).

The genus *Tupinambis*, which involves at least five species (Ávila-Pires, 1995, Fitzgerald et al., 1999), consists of a group of large carnivorous lizards, inhabitants of the South American plains east of the Andes (Presch, 1973). *Tupinambis merianae* and *T. rufescens* are the most southern representatives of the genus, and their populations reach the north part of the Patagonia (Cei and Scolaro, 1982). Both species have been conventionally used by indigenous communities of Argentina and Paraguay as a source of meat, fat, and leather (Donadio and Gallardo, 1984; Norman, 1987). At the present time, they are included in Appendix II of CITES, due to the excessive exploitation they suffer in the procurement of their hides (Chardonnet et al., 2002). This situation has led to a proposal of breeding these lizards in captivity for economic purposes (Mercolli and Yanosky, 1990; Noriega et al., 1996).

These lizards show a series of distinctive reproductive behaviors, of which territorial demarcation is a major characteristic (Mercolli and Yanosky, 1990; Fitzgerald et al., 1991; Noriega et al., 1996). Even though femoral glands seem to play an important part in the reproductive process, presumably by releasing pheromones, no studies have been done on the organization of these structures. With the present study, we sought to contribute data to this topic, performing a morphological analysis of the femoral glands of *Tupinambis merianae* with light and electron microscopy.

## MATERIALS AND METHODS

### Animals

This study was carried out using specimens of *Tupinambis merianae* from the breeding colony of the experimental farm at the Faculty of Agronomy and Zootecnia of the National University of Tucuman in Northern Argentina. The breeding stock consisted of groups of one male and five or six females, housed in open-air pens. The pens were surrounded by masonry walls 1.2 m high; each pen contained a shelter, provided shade, and allowed each individual an area of 2 $m^2$. The animals were fed ad libitum with a hatchery diet (Vega Parry and Manes, 2000).

The external aspect of the femoral glands was observed throughout the year in specimens of both genders. Changes in their appearance were associated with particular states of the reproductive cycle.

### Sampling of femoral glands

Groups of femoral glands of two adult male specimens were surgically removed during the mating season (October). The ani-

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mals, weighing 5.4 and 3.4 kg and measuring 52 and 43 cm in snout-vent length, were actively involved in territorial demarcation behaviors. After the animals had been sedated with Diazepam (2.5 mg/kg) and anesthetized with ketamine (25 mg/kg), sections of skin of 2.5×1 cm in area were removed from the thighs, each of which contained 8 or 9 glands. The skin samples were subsequently divided into fragments containing 2 or 3 glands each.

In some of the male specimens, the secretions were extracted with fine pliers for examination of their morphological characteristics, properties, and reaction to staining in toto with Sudan Red.

### Preparations for light microscopy

For histological examination, a group of glands was fixed in Duboscq-Brasil fixative (Langeron, 1949) for 48–72 hours, embedded in paraffin using standard techniques, and sectioned at 7-μm thickness. The sections were stained with hematoxylin-eosin and with Gallego's technique with formol (Langeron, 1949).

### Preparations for electron microscopy

Fragments of skin containing glands were fixed in half-concentration Karnovsky's fixative (2.5% glutaraldehyde, 2% formaldehyde, pH 7.4; Karnovsky, 1965) for 48 hours. For transmission electron microscopy (TEM), fragments of the glands were post-fixed in 1%, osmium tetroxide, pH 7.4, for 2 hours, and then were dehydrated in ethanol and acetone and embedded in Spurr resin. Ultrathin sections were stained with uranyl acetate and lead citrate (Venable and Coggeshal, 1965) and examined with a Zeiss 109 transmission electron microscope.

For scanning electron microscopy (SEM), whole glands were fixed in Karnovsky's fixative, dehydrated in ethanol and acetone, and critical-point dried. Specimens were sputter coated with gold and observed with a JEOL 35 CF scanning electron microscope.

## RESULTS

### General observations

Femoral glands (24 to 26 pairs) were counted in specimens of both genders of *Tupinambis merianae*. They appear as a row of scales with a central pore, placed ventrally in the thigh and precloacal area (Fig. 1A). Cylinders of secretion were observed only in male specimens and only during the mating period (Fig. 3A), and coincided with a characteristic territorial behavior that consisted of displacement, with a slightly wavy dragging of the pelvic girdle and hind legs on the ground.

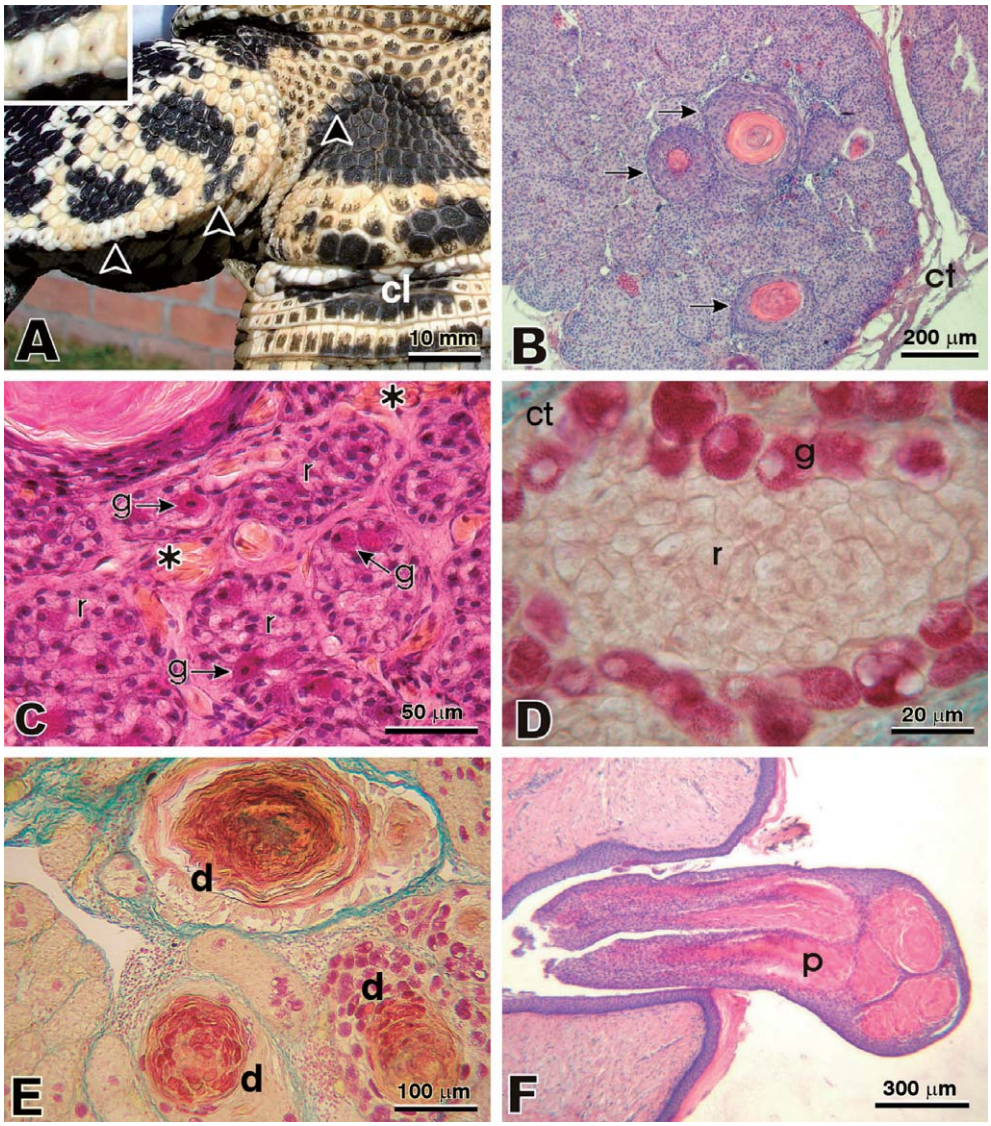


**Fig. 1.** Femoral glands in male specimens of *Tupinambis merianae*: direct and light-microscopic observations. **(A)** Pore-bearing scales corresponding to the femoral gland openings (arrowheads) in thigh and precloacal region. Insert: higher magnification of three pore-bearing scales. cl, cloaca. **(B)** Femoral gland lobe, with cross-sectioned ductules full of secretion (arrows); note the glandular parenchyma formed by numerous secretory units. ct, interlobular connective tissue. Stained with hematoxylin-eosin. **(C)** Group of adenomeres consisting of numerous cells containing clear, reticulated cytoplasm (r) and fewer strongly eosinophilic cells (g). Abundant vascularization is seen around the secretory units (asterisks). Stained with hematoxylin-eosin. **(D)** Cross-section of a secretory unit at its apex. In the center are clear reticulated cells (r) with a compact arrangement. At the periphery are round cells with a large vacuole and intensely stained cytoplasmic granules (g). ct, connective tissue. Gallego staining. **(E)** Cross-section of subsidiary ducts showing changes in the secretion. Below, two ducts (d) in which cells are entering the secretion, interspersed with layers of keratin. Above, a larger duct (d), with a secretion consisting of amorphous material and keratin layers. Gallego staining. **(F)** Longitudinal section of a secretion cylinder; note its conformation from partial secretions (p). Layers of epithelial cells surround the cylinder, and partial secretions laterally. The epithelium evident at the distal end of the cylinder is due to the obliquity of the section. Stained with hematoxylin-eosin.

This type of displacement, usually at the perimeter of the enclosures, caused rubbing of the thighs and cloacal region on areas of grass and bare soil. Some females occasionally simulated this type of movement, though rudimentarily and for brief moments. Replacement males introduced into the breeding pens engaged in tongue-flicking reactions and marked the trajectories of previous males.

Secretion cylinders were easily extracted from the femoral pores by using slim pliers. They showed resistance to manipulation, as well as flexibility. They did not fracture crosswise, although they could be incised lengthwise into smaller units. They stained lightly with Sudan Red, which may indicate the presence of lipidic material. We detected no odor.

**Observations of the femoral gland by light microscopy**

Each egg-shapped gland is located subcutaneously, measures approximately 3× 1.5 mm, and is lobed and composed of several secretory units. The secretion is transported through a series of internal ductules towards the main duct, which will carry it to the exterior via the pore (Fig. 1B, F). Histologically, the gland is formed of two cell types (Fig. 1C, D). The more abundant type consists of polyhedral shaped cells in a compact group, with clear, net-like cytoplasm. The other type comprises round eosinofilic cells with abundant granulation in their cytoplasm, after staining with Gallego's technique (Fig. 1D). These cells increase in number from the base to the apex of the gland, where they are located peripheral to the former type, and they also acquire conspicuous vacuolization (Fig. 1D).

The holocrine secretion comes from both cellular types. Initially, the cells can be easily distinguished, but they gradually lose integrity, becoming a semi-amorphous material alternating with concentric keratin layers (Fig. 1E). The partial secretions converge into a solid unified secretion that is released to the exterior through the gland's main duct (Fig. 1F). Both partial and total secretions are covered with additional layers of epithelial cells.

In addition to fibroblasts, melanophores, mastocytes, and collagen fibers, the connective tissue linked to the gland also shows profuse vascularization.

**Observations of the femoral gland by electron microscopy**

The reticulate cells examined with TEM appear as typical keratinocytes with thick bundles of tonofilaments (Fig. 2A). These cells seem to relate to each other by a wide stripe of plasmalemmal interdigitations, and they are connected by desmosomal unions (Fig. 2A, B). Rich deposits of

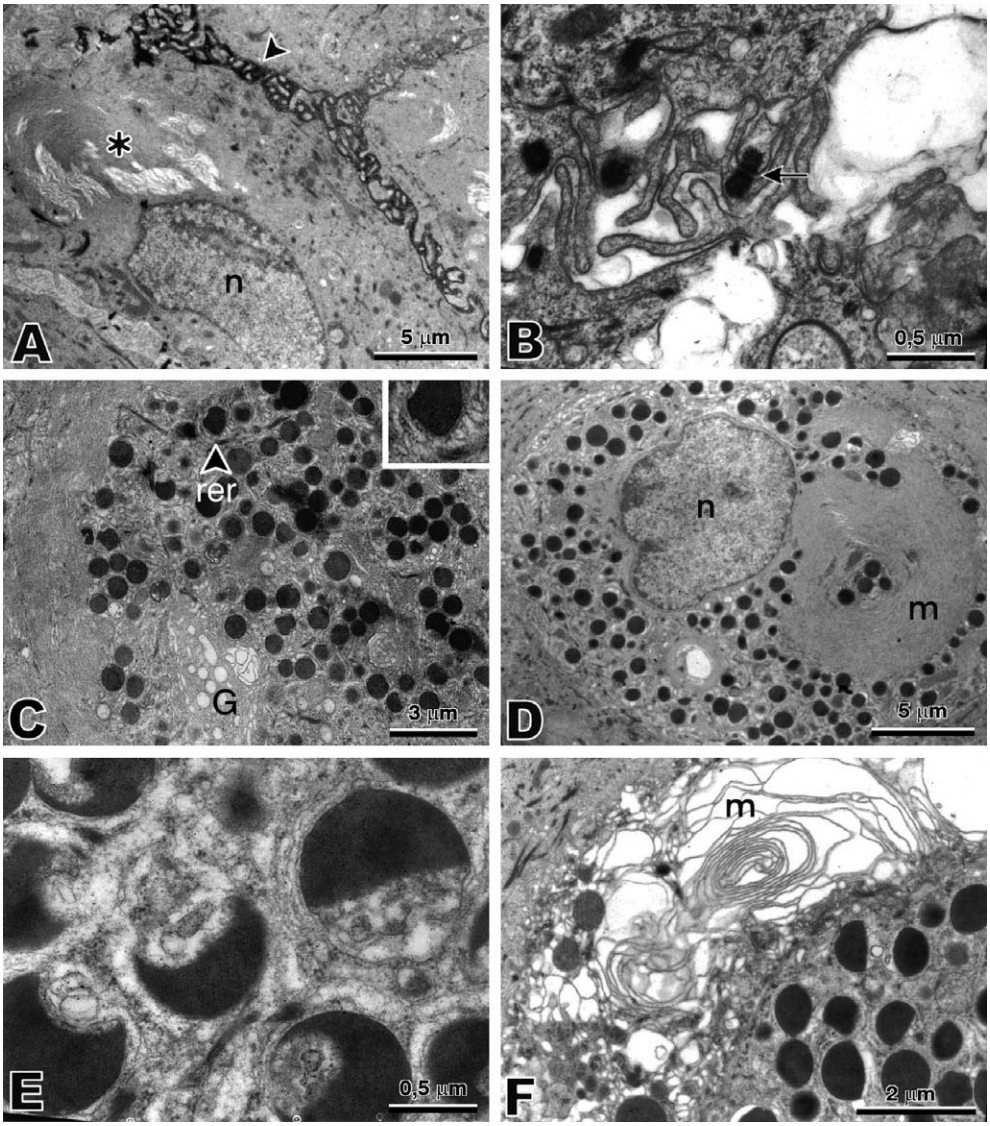


**Fig. 2.** Femoral glands in male specimens of *Tupinambis merianae*: observations with transmission electron microscopy. **(A)** Thick bundles of tonofilaments (asterisk) adjacent to the nucleus (n) in a keratinocyte-type cell. The extracellular space is occupied by an abundant accumulation of keratohyaline material (arrowhead). **(B)** Detail of a stripe of interdigitations between keratinocyte-type cells. The arrow indicates a desmosome. **(C)** Granular cell showing a prominent Golgi apparatus (G) and rough endoplasmic reticulum (rer). Insert: association of rer cisternae with a granule. **(D)** Granular cell showing electrodense granules and multilamellar bodies (m); note the rounded contour of the cell. **(E)** Granules showing vesicular contents. **(F)** Portion of a granular cell showing the deployment of membranous material (m).

electrodense keratohyalin material occur in the intercellular spaces (Fig. 2A). In the periphery of the secretory units, cells are joined to the basement membrane by hemidesmosomes.

The granular cells studied with TEM contain electrodense cytoplasmic granules of different sizes and which are surrounded by membranes (Fig. 2C, D, E). They also exhibit a well-developed Golgi apparatus and rough endoplasmic reticulum associated with the granules (Fig. 2C). In addition, they also contain voluminous bodies made of concentric, tightly coiled membranous layers (Fig. 2D). In some cases, all of these structures exhibit considerable changes in organization. The granules show less electrodensity, with apparent vesiculization of their content (Fig. 2E). The multilaminar bodies, in turn, display significant deployment (Fig. 2F).

Under SEM, the secretion cylinder appears to be covered by layers of epithelial cells and keratin (Fig. 3B, C). At higher magnification, granules similar to those in granular cells are also found free in the matrix (Fig. 3D).

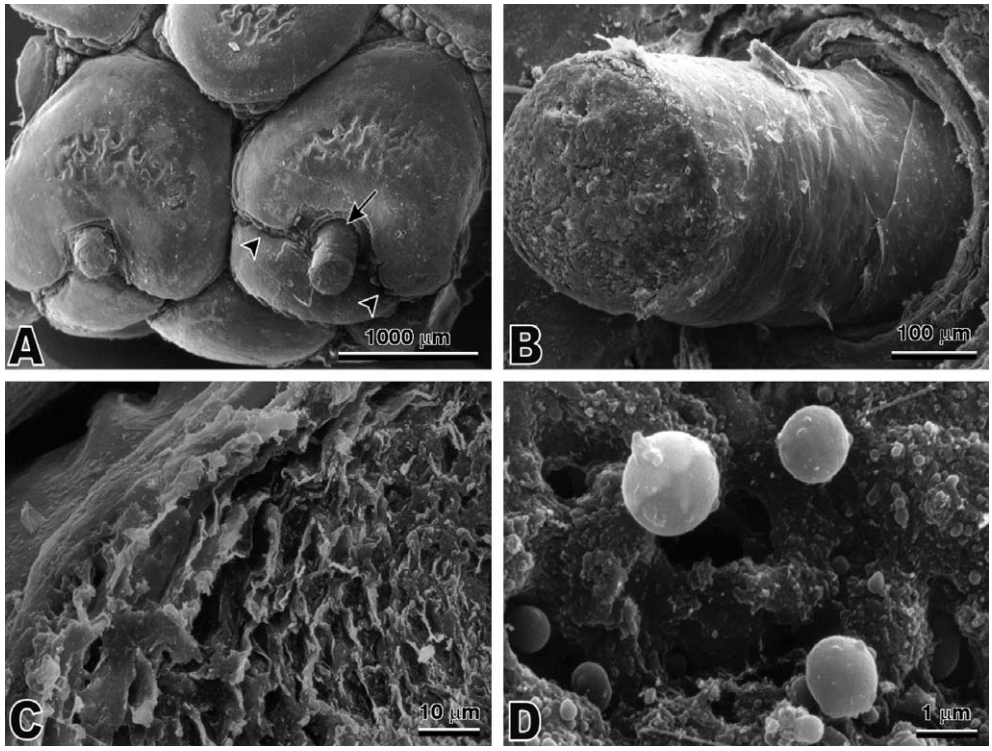


**Fig. 3.** Femoral glands in male specimens of *Tupinambis merianae*: observations with scanning electron microscopy. **(A)** Modified scales at the outlet of the femoral glands. In addition to a central pore occupied by a secretion cylinder (arrow), two grooves extend from the pore towards the caudal edge (arrowheads). **(B)** A secretion cylinder covered laterally by epithelial cell layers. In contrast, the rubbing area exhibits a rough surface of semiamorphous material. **(C)** Laminar organization at the margin of the secretion cylinder. **(D)** Free granules in the matrix of the secretion cylinder.

## DISCUSSION

The seasonal activity of *Tupinambis merianae* in temperate and subtropical environments, with hibernation periods of up to 6 months, restricts its main reproductive functions (copulation, egg-laying, hatching, and birth) to spring and the beginning of summer (Mercolli and Yanosky, 1990; Noriega et al., 1996). Breeding in captivity does not seem to affect this cycle, or the lizard's reproductive capacity, since several generations of specimens were obtained under these conditions.

Femoral glands, apparently involved in the production of semiochemical signals (Cooper et al., 1986; Cooper and Vitt, 1986; Cooper and Garskta, 1987), were observed to be active during the short, intense phase of sexual interaction that occurs around October (Noriega et al., 2002; Manes et al., 2007).

The presence of secretion cylinders in the femoral pores of adult males, and their absence in females, is in line with other aspects of sexual dimorphism observed for this species; male specimens are larger, with a wider head, better developed mandibular muscles, and scales in the form of cloacal buttons (Donadío and Gallardo, 1984; Mercolli and Yanosky, 1990; Fitzgerald et al., 1991; Noriega et al., 1996). Moreover, the apparent difference in glandular function between the sexes is not surprising, since the territorial behavior is distinctly a male characteristic (Mercolli and Yanosky, 1990, Noriega et al., 1996). During territorial demarcation, male individuals typically rub their thighs and cloacal region on the ground (Mercolli and Yanosky, 1990, Fitzgerald et al., 1991; Noriega et al., 1996). This behavior, which we also observed, abrades the secretion cylinders, leaving a trail of odor on the soil. Although there was no direct evidence of material deposition, the tongue-flicking reaction and the overlapping of territorial demarcations when some males were replaced by others indicate the presence of secretions on the substrate.

The discharge of secretion by *Tupinambis merianae* appears to be similar to that described for *Amphisbaena alba* (Jared et al., 1999), but different from that suggested for *Ameiva ameiva*, where the secretion seems to fragment when the animal is at rest, due to particular scales around the femoral pore (Imparato et al., 2007). A noteworthy morphological aspect that seems to support these differences is the presence of abundant keratin in the secretion cylinders of *Tupinambis merianae*, which makes the secretion far less prone to fragmentation compared to that of *Ameiva ameiva* (Imparato et al., 2007). Consequently, dispersion of the signal by the femoral glands of *T. merianae* can be considered an active process, associated with the particular mode of territorial demarcation of this species, in contrast to what happens with other lizards (Alberts, 1993; Jared et al., 1999; Imparato et al., 2007).

The most common cells, probably keratinocytes joined by wide bands of interdigitations and desmosomic bridges, appear to be involved in the formation of the corneal layers of the secretion. Even though these cells are rich in tonofilaments and keratohyaline material, their weak staining with Sudan Red may indicate lipidic material in the secretion. On the other hand, the granular cells found in the net of keratinocytes as isolated, non-epithelial elements may be assertively considered responsible for the semiochemical function of the gland. The granules in these cells, which may be reservoirs of pheromones, were observed free in the matrix of the secretion. As observed in *Ameiva ameiva* (Imparato et al., 2007), these granules may represent the smallest structure deposited by *Tupinambis merianae* during territorial demarcation; however, ultrastructural changes in the granules, similar to those described for *Ameiva ameiva* (Imparato et al., 2007), may entail changes in the final composition of the secretion. The granular cells also have multilaminar membranous structures, probably corresponding to the wide vacuoles observed by light microscope. Further study will be needed to fully understand the functional connotation of these changes and the connection between these structures.

Knowledge of the morphological organization of the femoral glands of *Tupinambis merianae* offers a starting point for analyzing semiochemical communication in this species, and for identifying the molecules involved.

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