

Oviductal Structure and Ultrastructure of the Oviparous Gecko, *Hemidactylus Mabouia* (Moreau De Jonnès, 1818)

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ABSTRACT

Lizards of the family Gekkonidae display a variety of reproductive patterns, as evidenced by the presence of viviparous and oviparous species. The species *Hemidactylus mabouia* is oviparous. We examined, in vitellogenic females, oviductal structure by light microscopy after routine histological and histochemical techniques, as well as by scanning and transmission electron microscopy. The oviduct is composed of four different regions: the infundibulum, which opens into the coelomic cavity and receives the oocyte released at the time of ovulation; the uterine tube, where sperm storage takes place; the uterus, which is responsible for the eggshell production; and the vagina, the final portion of the oviduct that leads to the cloaca. The oviductal structure of *H. mabouia* is similar to that of other oviparous lizard species and can be useful for morphological comparative analysis among reptile species. *Anat Rec*, 294:883–892, 2011. © 2011 Wiley-Liss, Inc.

Key words: oviduct; reproductive biology; Squamata; ultrastructure

The female reproductive system of reptiles includes ovaries and oviducts derived from the embryonic paramesonephric ducts (Wake, 1985). Oviducts are a pair of organs that are formed by morphologically distinct segments, which may have small variations between different species. Usually, the reptilian oviduct is divided into four or five regions called the infundibulum, uterine tube, isthmus, uterus, and vagina (Girling et al., 1998). The oviducts have very important functions for reproduction. The Gekkonidae family includes both viviparous and oviparous representatives (Girling et al., 1998). In oviparous species, such as *Hemidactylus mabouia*, a prominent function of the oviducts is eggshell production. In viviparous species, oviducts act in the formation of the placenta (Yaron, 1985; Stewart and Thompson, 1993, 1996; Blackburn, 1993a,b, 1998; Girling, 2002). In addition to provide a nurturing environment for the egg, specializations of the oviduct may also contribute to variation in the reproductive cycle of geckos. Some species of the family Gekkonidae store

sperm in specific oviduct regions, dissociating mating from fertilization.

Investigations on oviduct samples from *Hemidactylus* are well documented in the literature (Picariello et al., 1989; Murphy-Walker and Haley, 1996; Girling et al., 1997, 1998, 2000). Although there are ecological (Zamprogno et al., 1998; Rocha et al., 2002) and

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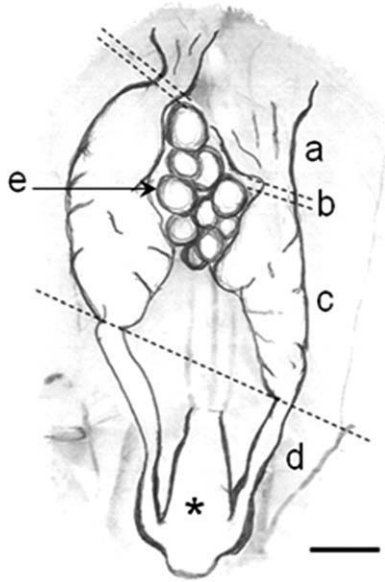


Fig. 1. Scheme of the female reproductive tract and cloaca of the *H. mabouia*. a, Infundibulum; b, uterine tube; c, uterus; d, vagina; e, ovaries. * cloaca. Bar: 2 mm.

behavioral studies (Vanzolini, 1978; Vitt, 1986) on the species *H. mabouia*, the oviduct morphology of this species has not yet been described. Moreover, *H. mabouia* is a commonly found species, which makes it useful for morphological comparative analysis among reptile species. This study describes the histology and ultrastructure of each segment of the *H. mabouia* oviduct during the vitellogenic period.

MATERIAL AND METHODS

Specimens of *H. mabouia* were collected for this research under Brazilian Institute of Environment and Renewable Natural Resources license number 10504-1. Their carcasses are lodged at the Museum of Zoology Joao Moojen, Federal University of Viçosa (UFV), Brazil. The whole experiment was conducted in accordance with the ethical principles for the laboratory use of animals published by the Brazilian College of Animal Experimentation.

Twelve adult vitellogenic females of *H. mabouia* were captured from human habitations in the cities of Viçosa-MG and Cambuci-RJ, Brazil. The follicles of vitellogenic females were firm and spherical (approximately 6 mm) and could be identified by gentle palpation of the abdomen and confirmed upon dissection. The late vitellogenesis was defined when ovarian follicles reach a diameter greater than 7 mm. The snout-vent lengths of the *H. mabouia* specimens used in this study varied between 51.4 and 57.2 mm in accordance with the criteria established by Rocha et al. (2002), the specimens were characterized as adults.

The identification and collection of the oviducts were performed *in situ* by a medial longitudinal incision in the animals' ventral regions in saline solution. Samples of each oviductal region were immediately fixed in Carson's solution (Carson et al., 1973) for at least 24 hrs, at

room temperature for the light microscopy. The samples were dehydrated in increasing alcohol concentrations with changes every 30 min. After dehydration, the material was embedded in glycol methacrylate (Historesin, Leica). The samples were sectioned (0.5 and 2 μm) using an automatic microtome (RM-2155, Leica) with glass knives. The sections were stained in 1% toluidine blue (TB) or with the following histochemical techniques: periodic acid-Schiff (PAS) and Alcian blue pH 2.5 (AB) for the detection of neutral and acid glycoconjugates, respectively; Nile blue sulfate (NB) (Bancroft and Steven, 1996) for acid and neutral lipid detection; and xylydine ponceau (XP) for protein detection (Mello and Vidal, 1980). After treatments, the material was mounted on glass slides with Entellan[®] (Merck). The tissues were analyzed using an Olympus BX-60 microscope with a Q-Color 3 (Olympus) digital camera in the Insect Cytogenetics Laboratory - Federal University of Viçosa, Brazil.

Scanning and Transmission Electron Microscopy

For ultrastructural analysis, tissue fragments were cut into small pieces ($\sim 1 \text{ mm}^2$) and separated for use in scanning and transmission electron microscopy. Tissues were fixed in Karnovsky's solution for 4 hrs. Tissues for scanning electron microscopy were dehydrated through an ethanol series and critical point dried in liquid CO_2 . Afterward, the samples were mounted and coated with a fine layer of gold in an SCA 010 sputter coating attachment and viewed under a LEO VP1430 scanning electron microscope. Tissues for transmission electron microscopy were postfixed in 1% buffer osmium tetroxide/cacodylate for 1 hr, dehydrated through an ethanol series and infiltrated with Epon resin. Ultrathin sections were cut using the ultramicrotome (DuPont-Sorvall, Porter-Blum MT2-B) and stained with uranyl acetate (2%) and lead citrate (0.2%). Samples were viewed with an EM 109 - Zeiss transmission electron microscope in the Nucleus of Microscopy and Microanalysis at UFV.

RESULTS

The oviducts are located in the coelomic cavity of females. Usually, the left oviduct's anterior extremity is located posterior to the right. Each oviduct of *H. mabouia* can be divided anatomically into four regions: the infundibulum, the uterine tube, the uterus, and the vagina (Fig. 1). The vagina is the final portion of the oviduct that opens into the cloaca. The cloaca is, in turn, an area composed of three portions where the digestive, genital, and urinary systems empty. The oviduct can also be divided in three tissue layers in cross section. The mucosa, the innermost layer, consists of an epithelial layer plus the *lamina propria*. Under the mucosa is the muscularis, which consists in one or two smooth muscle layer. The oviduct is enclosed within the serosa, a continuation of the peritoneum.

Infundibulum

The infundibulum is the most anterior segment of the oviduct, which is organized in several longitudinal folds in the coelomic cavity. It possesses an ostium, through which oocytes enter after ovulation. The infundibulum's

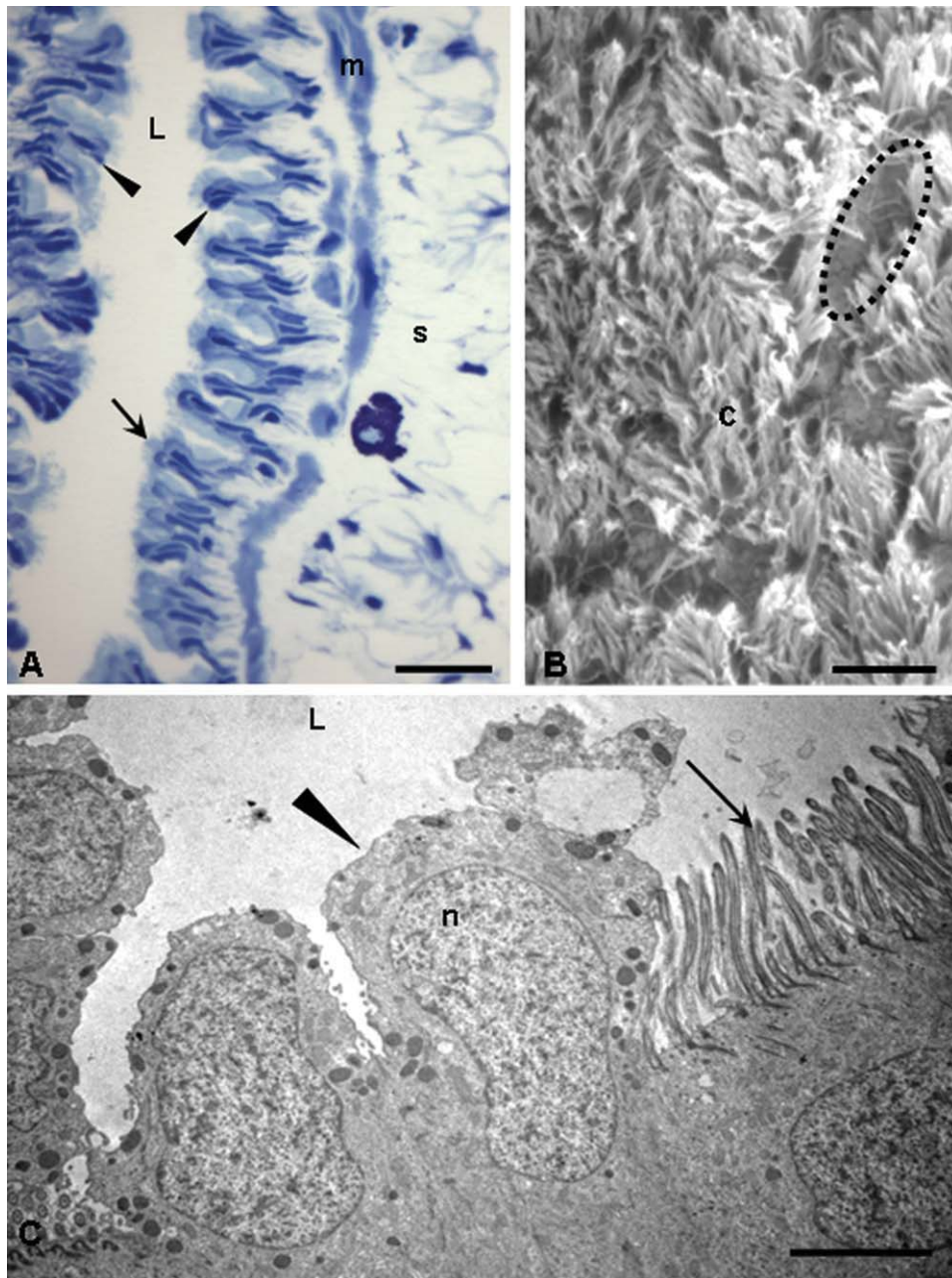


Fig. 2. Light microscopy (A), scanning (B), and transmission (C) electron microscopy of the infundibulum of the *H. mabouia*. A and C, Ciliated (arrow) and nonciliated cells (arrow heads). B, Ciliated (c)

and nonciliated cells (circle). Note the nucleus and apical cytoplasm of nonciliated cells protruding into the lumen. Bars: A, 20 μm ; B, 5 μm ; C, 3 μm . c, cilia; L, lumen; m, smooth muscle; n, nucleus; s, serosa.

mucosa is lined by a simple epithelium that contains predominantly columnar ciliated cells with few nonciliated cells (Fig. 2A,B). The nuclei and apical cytoplasm of the nonciliated cells protrude into the lumen (Fig. 2C). The *l. propria* is thin, composed of loose connective tissue and lacking in glands. Underneath the mucosa, there is a thin layer of smooth muscle, one or two cells thick. The smooth muscle cells present irregular surfaces, and they have a no clearly defined orientation in the layer. The serosa, formed by simple squamous epithelium, includes

the muscularis. Some nonciliated cells possess apical granules that stained positively with AB for acid glycoconjugates but did not stain with PAS for neutral glycoconjugates.

Uterine Tube

The uterine tube is a small region between the terminal portion of the infundibulum and the beginning of the uterus. Its epithelium contains both ciliated and

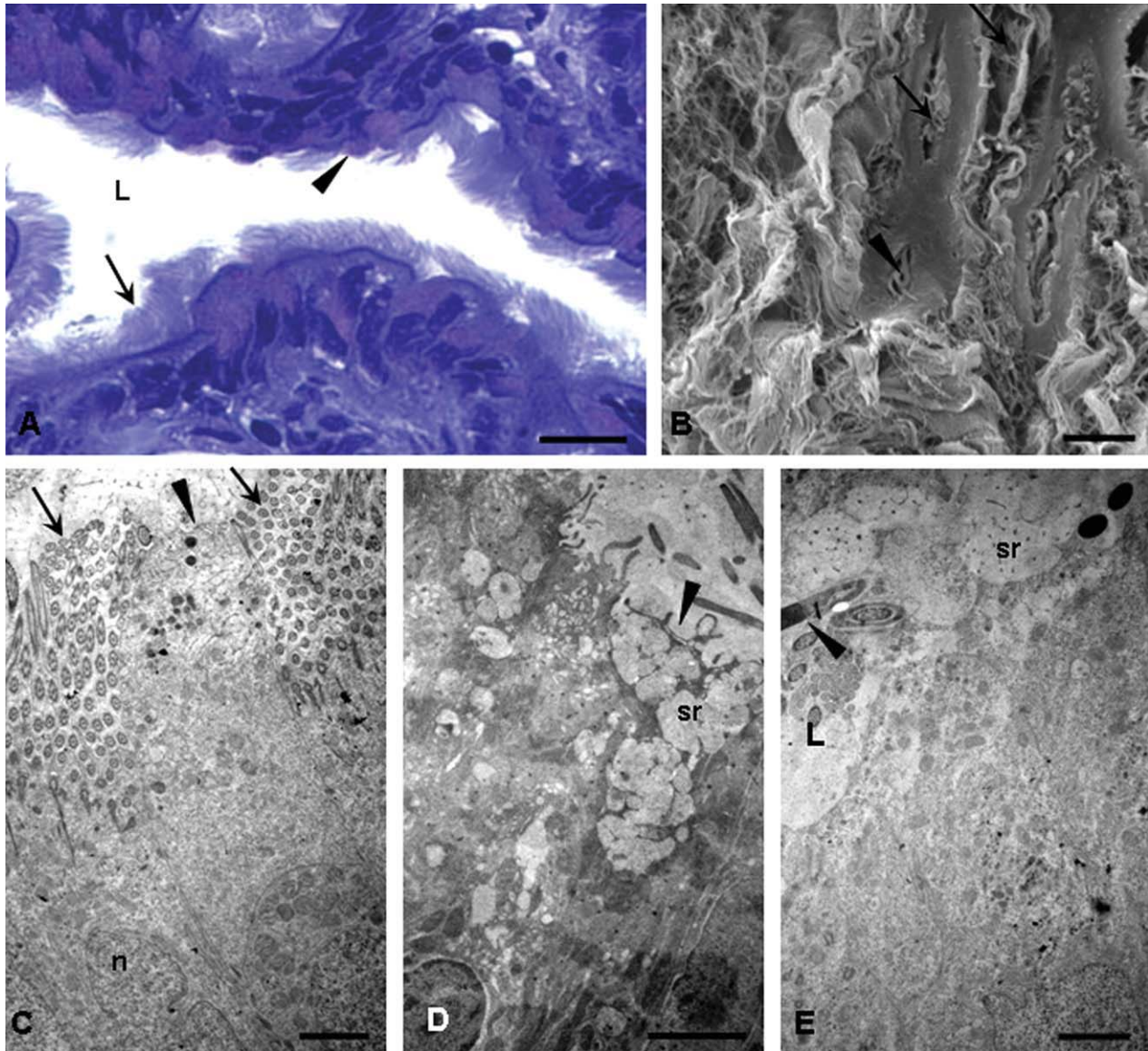


Fig. 3. Light microscopy (A), scanning (B), and transmission (C–E) electron microscopy of the uterine tube of the *H. mabouia*. A, Ciliated cell (arrow) in a crypt and secretory nonciliated cells (arrow head). B, Sperm storage (arrow head) in the crypts (arrows). C, Ciliated (arrows)

and secretory cells (arrow head). D, Secretory cell (arrow head). E, Sperm cell (arrow head) and secretion into the lumen. Bars: A and B, 10 μ m; C–E, 2 μ m. L, lumen; n, nucleus; sr, secretion.

nonciliated secretory cells (Fig. 3A,C,D). The secretions of these cells are metachromatic when stained with TB. In addition, the nonciliated cells stained positively with PAS and AB, indicating the presence of neutral and acid glycoconjugates in the secretions. The coating epithelium penetrates into the *l. propria* for originating crypts, which are elongated branched tubules that communicate with the lumen via ducts. These ducts contain both ciliated and nonciliated secretory cells. Some crypts contained many stored sperm cells (Fig. 3B,E).

Uterus

The uterine wall has numerous folds. From an anatomical point of view, it corresponds to the thickest

area of the whole oviduct. The uterine epithelium contains both columnar ciliated and nonciliated cells with elliptic nuclei (Fig. 4A,C). The nonciliated cells are of two types; one type possesses an arched rough surface, while the other has narrow apices with flat surfaces (Fig. 4B). Semithin sections of the uterus late in the vitellogenic period illustrated that epithelium is simple. In late vitellogenic females, the volume of the epithelial cells was significantly increased compared with early vitellogenic females. The infranuclear area of the epithelial cells is rich in granular material when stained with TB. Beneath the epithelial layer, there is a profusion of small blood vessels forming an extensive plexus (Fig. 4A,D). The apical border of the nonciliated cells stained positively for neutral and acid glycoconjugates.

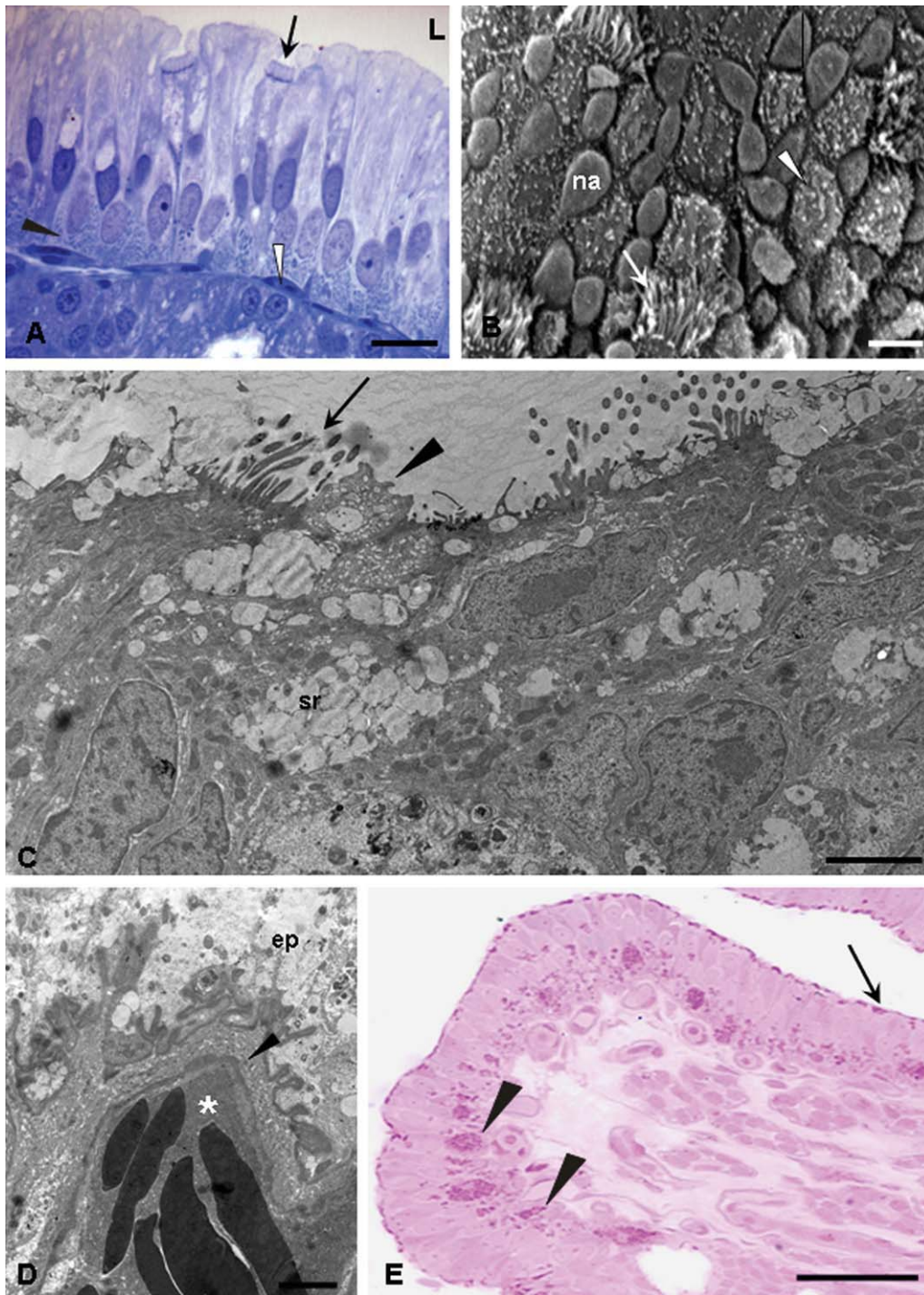


Fig. 4. Light microscopy (A and E), scanning (B), and transmission electron microscopy (C and D) of the uterus of the *H. mabouia*. A, Semithin section showing ciliated cells (arrow), granular material under the nucleus (arrow heads), and blood vessels (white arrow heads). B, Figure showing three different types of cells: ciliated (arrow), narrow apices (na), and rough surface (arrow heads). C, Ciliated (arrow), and

nonciliated cells (arrow head). D, Section showing the proximity of the blood vessel (asterisk) and the basal lamina (arrow head). E, Positive staining for PAS at the surface of the epithelial cells (arrow) and in granules in the underlying connective tissue (arrow heads). Bars: A, 10 μ m; B and E, 20 μ m; C and D, 3 μ m. ep, epithelium; L, lumen; sr, secretion.

Granules positive for neutral and acid glycoconjugates can be seen in the connective tissue underlying the epithelium (Fig. 4E). The uterine epithelial

cells did not show positive reactions with the histochemical techniques used for the detection of proteins and lipids.

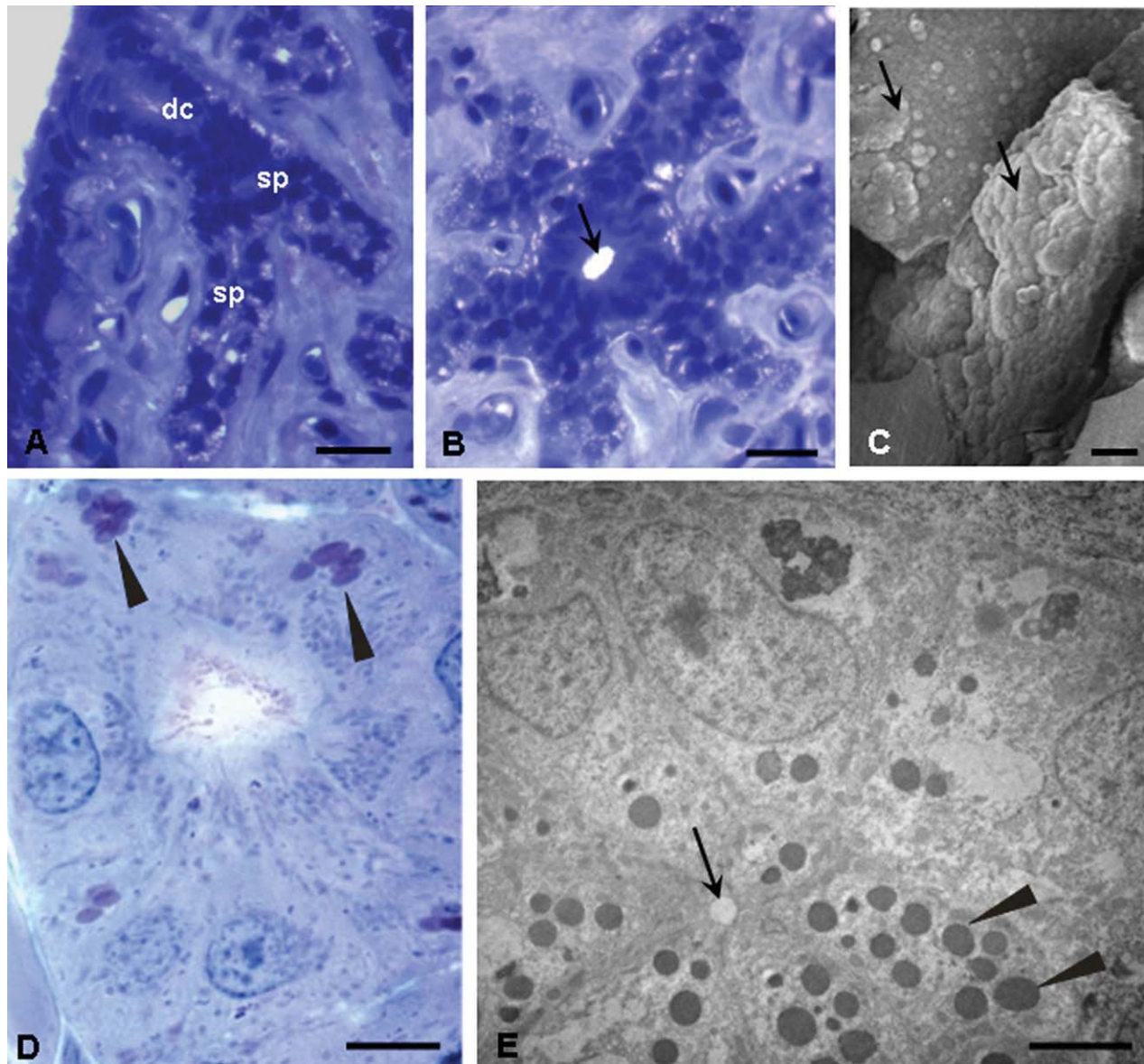


Fig. 5. Light microscopy (A, B, and D), scanning (C) and transmission electron microscopy (E) of the uterine glands of the *H. mabouia*. Note in A, the duct (dc) and the secretory portion (sp) and in B, note the duct lumen (arrow). C, Base of the glands showing the external surface of the secretory portion (arrows). D, Positive reaction to the

histochemical test NB, evidencing the presence of neutral fats in the base of the secretory cells. E, Section showing secretory granules (arrow heads) and the lumen (arrow). Bars: A and B, 20 μm ; D, 10 μm ; E, 5 μm . C, 40 μm . dc, duct; sp, secretion portion.

The uterus is the only truly glandular region of the oviduct. The tubular branched glands of the uterus (Fig. 5A–C) are also known as eggshell glands. The glandular cells have nuclei with unpacked chromatin (Fig. 5D,E). These cells reacted negatively to histochemical techniques for the detection of glycoconjugates and proteins. However, a positive reaction for neutral fats was observed in granules located below the nucleus of some cells and in the lumen in the sample from late in the vitellogenic period (Fig. 5D).

Vagina

The vagina is the final region of the oviduct. The vaginal mucosa is organized into folds that increase in size

as they approach the cloaca. The morphological characteristics of the vaginal wall facilitate its division into two segments: the anterior segment that lies adjacent to the uterus and the posterior segment that leads to the cloaca. Both portions of the vagina are surrounded by a muscular layer that thickens as it approaches the cloaca. The anterior segment of the vagina (anterior vagina) is lined by a simple columnar epithelium containing ciliated and nonciliated cells (Fig. 6A–D). Ciliated cells predominate in the whole extension of the anterior vagina (Fig. 6A). Two types of nonciliated cells were observed, one of which is typically secretory (Fig. 6D). Its secretion is metachromatic when stained with TB and

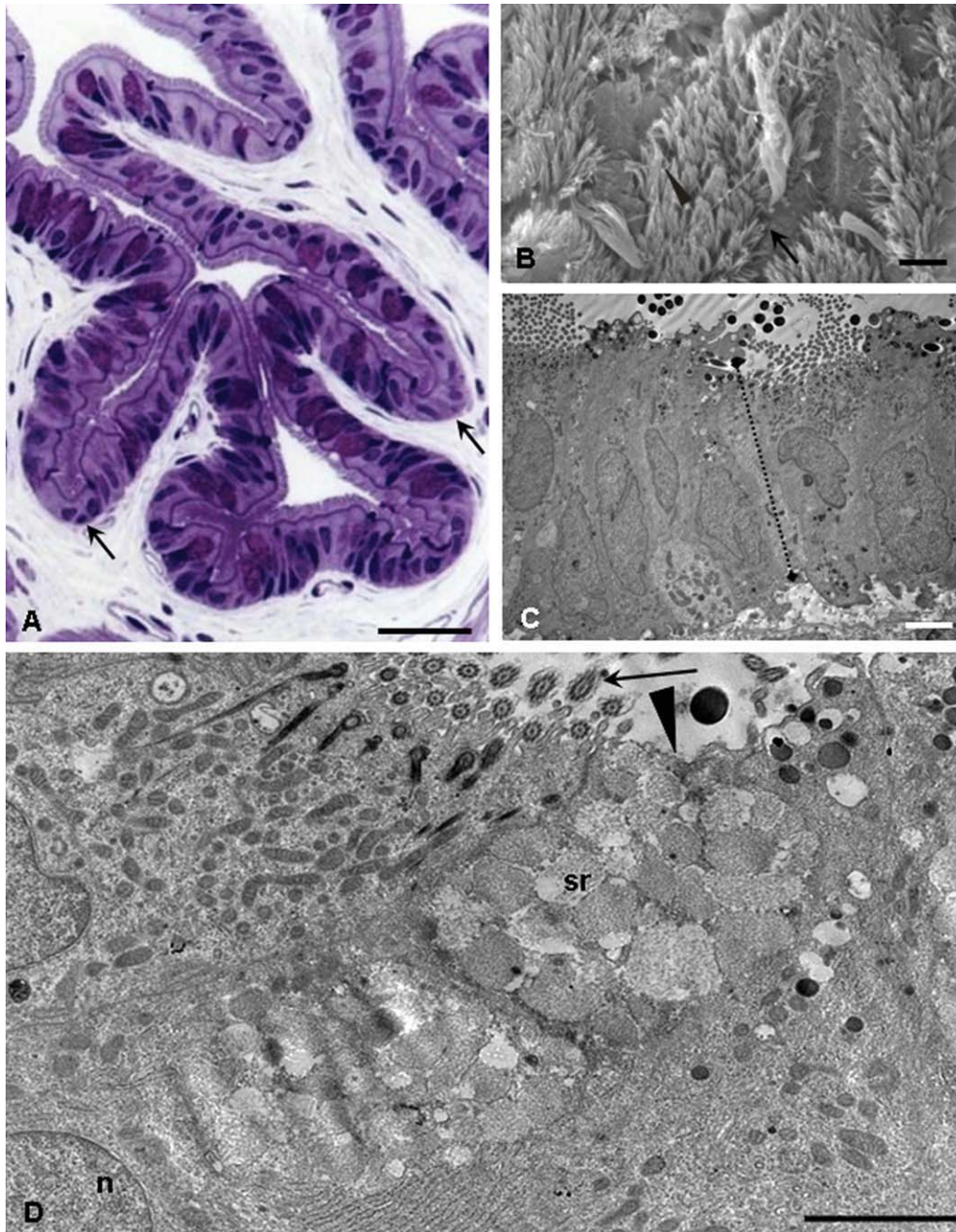


Fig. 6. Light microscopy (A), scanning (B), and transmission electron microscopy (C and D) of the anterior vagina of the *H. mabouia*. A, Columnar simple epithelium covering several pleats (arrows). B, Ciliated (arrow

head) and nonciliated cells (arrow). C, Section showing the simple epithelium. D, Ciliated (arrow) and nonciliated secretory cells (arrow head). n, nucleus; sr, secretion. Bars: A, 15 μ m; B, 5 μ m; C, 3 μ m; D, 2 μ m.

stained positively for neutral and acid glycoconjugates (Fig. 7A). This type of cell prevails close to the transition between the anterior and the posterior vagina (Fig. 7B). The posterior vagina possesses stratified epithelium composed of nonciliated cells (Fig. 7C). Its secretion gave a slightly positive reaction to the AB histochemical technique (for acid glycoconjugates) and did not stain with PAS (for glycoconjugates) or XP (for proteins).

DISCUSSION

The terminology as well as the anatomical and histological division of reptilian oviducts vary among authors, mainly in interspecific comparisons. Among the geckos, in the species *Hemidactylus turcicus*, *Saltuarius wyberba*, *Hoplodactylus maculatus*, *Hoplodactylus duvauvelii* (Girling et al. 1998), and *Tarentola mauritanica* (Picariello et al. 1989), the oviduct can be divided into five regions,

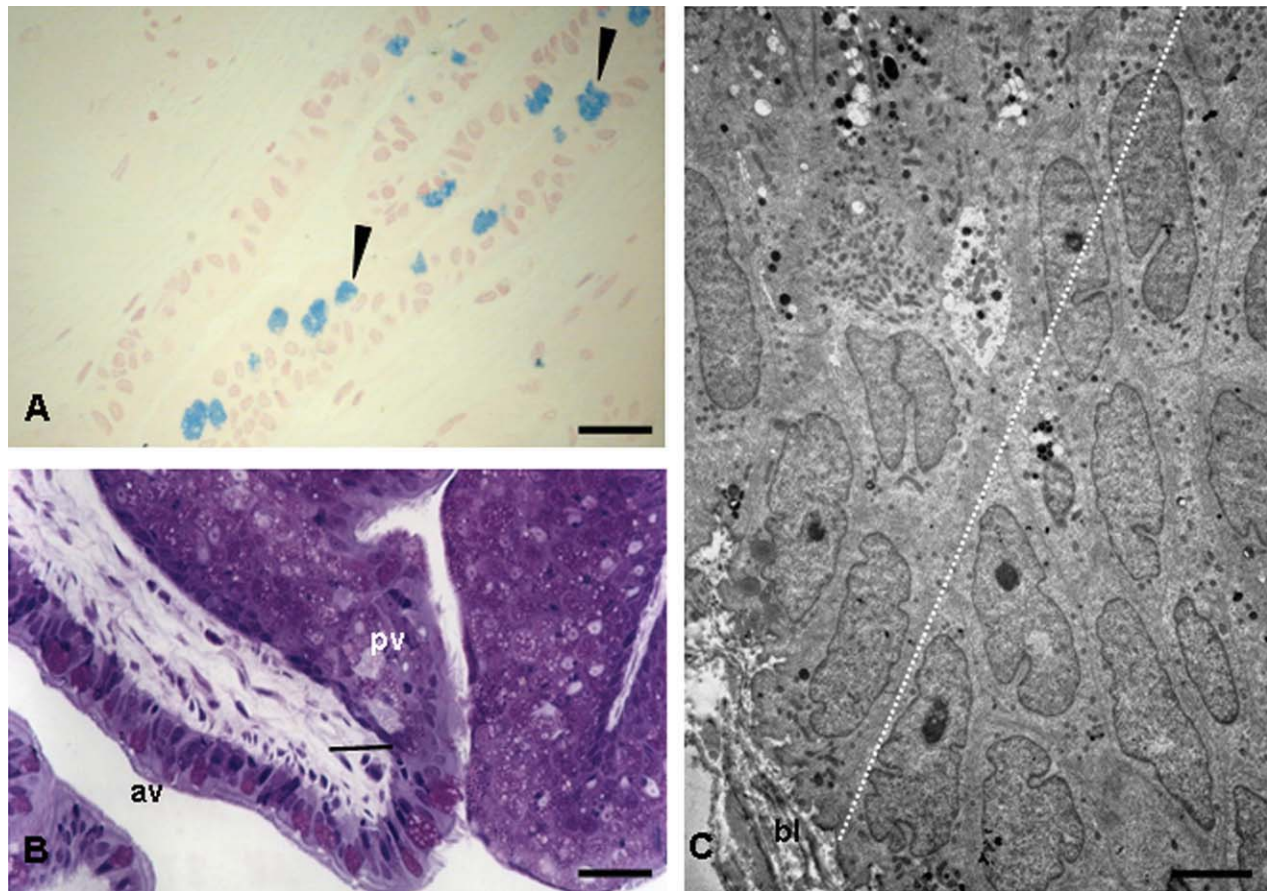


Fig. 7. Light microscopy (A), scanning (B) and transmission electron microscopy (C) of the vagina of the *H. mabouia*. A, Anterior vagina. Note the secretion of the nonciliated cells stained positively for AB (arrow heads). B, Transitional area of the vagina (arrow), showing the

anterior (av), and the posterior vagina (pv). C, Posterior vagina. Section showing the stratified epithelium. Bars: A and B, 10 μ m; C, 3 μ m. av, anterior vagina; pv, posterior vagina; bl, basal lamina.

namely the infundibulum, uterine tube, isthmus, uterus, and vagina. In *H. mabouia*, oviducts were anatomically divided into four areas, and the region of the isthmus, which, according to Girling et al. (1998, 2002), is a small aglandular region between the uterine tube and uterus, was not observed. A similar type of organization was observed by Guillette et al. (1989) in other families, such as the Crotaphytidae (*Crotaphilus collaris*) and Scincidae (*Plestiodon obsoletus*). The region denominated as uterine tube in *H. mabouia* has been described as posterior infundibulum in other Squamata, such as in the lizard *Calotes versicolor* (Kumari et al., 1990) and the snake *Seminatrix pygaea* (Sever et al., 2000).

The infundibulum of *H. mabouia* presents nonciliated cells that protrude into the lumen during the vitellogenic period. This type of cell was also observed in the infundibulum of *H. turcicus* by Girling (2002).

In spite of the importance of the albumen in the embryonic development of some species of reptiles and birds, the eggs of the Squamata order have lack the albumen layer (Cordero-López and Morales, 1995 and Sever e Hamlett, 2002). In this study, consistent with previously reported, the aforementioned protein was not observed using XP staining. The observation of noncili-

ated cells in the infundibulum of *H. mabouia* by scanning electronic microscopy was hindered by the presence of a great amount of cilia, as was also the case in *Lampropholis guichenoti* (Adams et al., 2004). However, nonciliated cells are evident under light microscopy and transmission electron microscopy.

The uterine tube of *H. mabouia* is a discrete region; however, it cannot be considered only a transitional region due to the significant histological differences between the uterine tube, the infundibulum, and the uterus. Moreover, the uterine tube is the region where sperm storage occurs, and this is perhaps one of the most important functions of the uterine tube in *H. mabouia*. The storage of sperm was previously observed and has been described in many species of lizards (Adams and Cooper, 1988; Murphy-Walker and Haley, 1996; Girling et al., 1997; Blackburn, 1998; Eckstut et al., 2009), snakes (Halpert et al., 1982; Birkhead, 1993; Sever and Ryan, 1999; Sever and Hopkins, 2004; Siegel and Sever, 2007), chelonians, and crocodiles (Girling, 2002). The storage is essential in some species due to the asynchronous reproductive cycle of males and females (Murphy-Walker and Haley, 1996). Moreover, the sperm storage allows copulation to be independent from the process of

fertilization (Girling, 2002), which can explain the capacity of *H. mabouia* females to lay eggs throughout the year (Vitt, 1986; Anjos and Rocha, 2008; Nogueira, 2008). The nonciliated epithelial cells of the uterine tube of *H. mabouia* stained strongly when submitted to the histochemical techniques for neutral and acid glycoconjugates, indicating secretory function, as was also observed in other lizard species (Girling, 2002) and for the snake *S. pygaea* (Sever and Ryan, 1999). These secretions are related to protection and lubrication of epithelial surfaces, which facilitates the passage of the oocyte and the egg through the oviduct (Botte, 1973; Girling, 2000; Sever et al., 2000).

The uterine epithelium of *H. mabouia* is lined by columnar ciliated and nonciliated cells, as it is in other oviparous lizards (Guillette et al., 1989; Picariello et al., 1989; Palmer et al., 1993; Perkins and Palmer, 1996; Girling et al., 1997, 1998, 2000; Girling, 2002; Adams et al., 2004). Despite the increase in height observed in uterine epithelial cells late in the vitellogenic period, there is no evidence of secretion during the vitellogenic period. Girling et al. (2000) relate an increase in the size of epithelial cells in the presence of estradiol in the species *H. turcicus*.

The presence of numerous uterine glands in *H. mabouia*, probably responsible for eggshell secretion, was also observed in other oviparous lizards of the family Gekkonidae (Girling et al., 1998; Guillette et al., 1989; Palmer et al., 1993). In viviparous species of Squamata, the number of glands is much reduced (Corso et al., 2000; Sever et al., 2000). In spite of the well-known secretory function of the uterine glands (Packard and DeMarco, 1991), they reacted negatively to the techniques for glycoconjugate and protein identification in the vitellogenic period of *H. mabouia*, as observed in *H. maculatus* and *H. turcicus* (Girling et al., 1997; Girling et al., 1998). Neutral lipids were found in glands of one of samples analyzed in *H. mabouia*, and similar results were observed in viviparous *S. pygaea* snake (Sever et al. 2000). The secretory nature of these glands is probably modified in function by small hormonal alterations during the sexual cycle.

According to Sánchez-Martínez et al. (2007), the vagina in Squamata does not show consistent differences in its morphology that justify its division into regions, and the differences are only present in response to sexual cycle phases. Adams and Cooper (1988) studied the vaginal morphology of the lizard *Holbrookia propinqua* and identified three uniformly ciliated regions that they characterized as anterior, middle, and posterior. In *H. mabouia*, the division into two regions is clearly demarcated by the epithelial transition between the anterior and posterior vagina; however, the posterior region does not present cilia.

An increase in the number and size of folds was observed in the vaginal mucosa of *H. mabouia* and was observed in *Sceloporus woodi* (Palmer et al. 1993). A different arrangement in which the mucosa folds become reduced in size as they approach the cloaca was observed in other lizards (Bott, 1973; Girling et al., 1997; 1998).

The vagina of *H. mabouia* does not possess crypts in the thin connective tissue constituting its *l. propria*. This observation suggests that sperm storage does not occur in this region.

This work described, for the first time in *H. mabouia*, aspects of the morphology and histochemistry of the oviduct, providing data that confirm similarities among other lizards that have been studied.

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