

# Histology and Histochemistry of the Oviduct of the Neotropical Tortoise *Phrynops geoffroanus* (Schweigger, 1812)

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## Abstract

*Phrynops geoffroanus* is specie of reptile of the Testudines order, popularly known as Geoffroy's side-necked turtle. As is the case of other representatives of this order, this turtle is also oviparous, meaning the females lay their eggs in the environment during reproduction. This work describes the morphology of the oviduct of *P. geoffroannus*, observed through light microscopy, contributing to the understanding of the reproductive biology of this species and of other reptiles. The oviducts of Geoffroy's side-necked turtle were sectioned and processed according to the routine histological techniques and were submitted to histological and histochemical methods (HE, Mallory's Trichrome, Ponceau Xylidine, AB pH 2.5, PAS). The oviduct of this turtle is composed of five regions: the infundibulum, which receives the oocytes released at the moment of ovulation; the uterine tube (magnum), a spiral-shaped region that produces albumin; the isthmus, a transition region; the uterus, where the egg shell is produced; and the vagina, the final part of the oviduct, which leads to the cloaca. The structure of the oviduct of *P. geoffroannus* is similar to that of other species of oviparous reptiles and can be used for phylogenetic morphological comparisons.

**Keywords:** Light microscopy; Morphology; Testudines; Infundibulum; Uterine tube; Isthmus; Uterus; Vagina

## Introduction

Reptiles form a prominent group in almost all terrestrial assemblages, and there are currently 9,596 known species [1]. Reptiles have suffered great losses throughout 38 their history, and of the previously existing orders, only the following four remain: Testudines, consisting of land, freshwater and sea turtles; Crocodylia, consisting of 40 caimans, crocodiles, alligators and gharials; Rhynchocephalia consisting of tuataras; and 41 Squamata consisting of snakes, lizards and amphisbaenians [2-6].

The order Testudines is subdivided into two suborders. The first suborder, Cryptodira (crypto: hidden; dire: neck), is divided into three superfamilies, Testudinoidea, Trionychoidea and Chelonioidea, that include sea, freshwater and land turtles found mostly in the Southern Hemisphere, terrestrial and aquatic species found in South America, and terrestrial species found in Africa. The second suborder, Pleurodira (pleuro: side), is divided into two families: Chelidae and Pelomedusidae, both of which are found only in the Southern Hemisphere [4,7].

Of all of the Chelidae family members, *Phrynops geoffroanus* has the broadest geographic distribution. The species is widely distributed in neotropical regions (Colombian Amazon, Venezuela, the Guyanas, Uruguay, northern Argentina and Brazil from north to south). Similar to other turtles, these species are oviparous with internal fertilization and have a reproductive system adapted to this condition [8,9].

In general, the reptilian female reproductive system consists of a pair of ovaries and oviducts, which lead the egg from ovulation to oviposition and/or parturition and provide the site of fertilization and sperm storage. In oviparous individuals, the oviduct actively participates in the production of the shell surrounding the egg, while in viviparous individuals; the oviduct participates in the formation of the placenta [10]. In reptiles, the term 'oviducts' designates the structures derived from the Müllerian ducts in the embryonic period [11]. In general, the oviduct can be anatomically divided into five regions. In

the rostro-caudal direction, these regions are as follows: infundibulum, uterine tube (magnum), isthmus, uterus and vagina [10].

The structure and function of the reptilian oviduct are poorly studied, particularly in sea turtles. Brazilian studies regarding the reproductive biology of *P. geoffroanus* primarily involve descriptions of reproductive behaviour and nesting; no studies have been involved the histological description of the female reproductive system. Therefore, the present study describes the microscopic anatomy of each segment of the *P. geoffroanus* oviduct, as revealed by histological and immunohistochemical techniques. In addition to enabling phylogenetic studies via morphological comparisons with other reptiles, the results described in this report should significantly improve our understanding of the reproductive biology of this species.

## Materials and Methods

Four adult female specimens of *P. geoffroanus* were used in this study. These were stored in liquid medium (10% formaldehyde) and deposited in the Herpetological Collection of the Department of Zoology at the Universidade Federal de Juiz de Fora - UFJF, Minas Gerais. Oviduct fragments of these specimens were stored, labeled and subsequently referred to the Laboratory of Histology and Embryology at Universidade Federal Rural of Rio of Janeiro.

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The organs were cut into cross sections at the UFRRJ Laboratory of Histology and Embryology and processed through a routine histological method, which included the following steps: dehydration (in graded series of ethanol from 70% to 100% (v/v)), diaphanization in xylol and impregnation with and embedding in paraffin. Subsequently, 5 µm thick sections were placed on slides, which were previously treated with poly L-lysine (Sigma-Aldrich, Inc.), to promote better adhesion of the sections to the slides. The sections were stained using histological methods, including Hematoxylin-Eosin (HE), for histological description of the organ Mallory's trichrome, for differentiation between collagen and muscle fibers, to evaluate the morphological pattern of muscle fibers and Xylidine Ponceau for total protein staining and to examine the oviduct architecture of *P. geoffroanus* under the light microscope [12]. The Periodic Acid-Schiff (PAS) and Alcian Blue (AB) pH 2.5 histochemical methods were used for detecting neutral and acidic Glycoconjugates (GCs), respectively. All slides were mounted with Entellan (Merk) following specific staining.

All slides were examined, and photomicrographs were taken, using a Sony Cybershot DSCW 230 digital camera coupled to an Olympus BX 40 microscope.

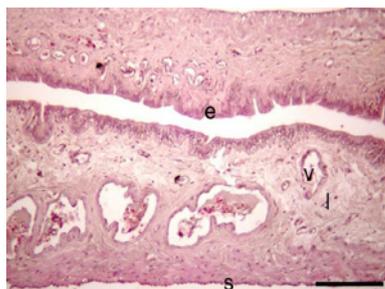
## Results

*P. geoffroanus* has a left oviduct and a right oviduct, and both are morphologically developed and physiologically functional. The oviducts begin near the ovaries and end in the cloaca and are dorsally located on each side of the body. Microscopic analysis of these oviducts has shown that each of these tubes can be subdivided into five histologically distinct regions from the ovary toward the cloaca. These tubes consist of the infundibulum, the uterine tube (magnum), the isthmus (transition region), the uterus and the vagina.

Histologically, the oviduct of the Geoffroy's toadhead turtle is organized into three distinct layers from the lumen to the coelom, as follows: mucosa, muscle and serosa. The mucus layer comprises two functionally related layers, the epithelium and the highly vascularized lamina propria, formed by connective tissue. The muscle layer is composed of smooth muscle fibers arranged into one or more layers according to the specific oviduct region, and the serosa consists of a thin layer of connective tissue lined by a single layer of squamous epithelium.

### Infundibulum

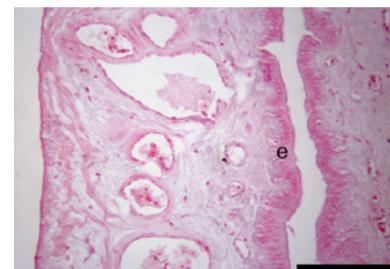
The infundibulum, the most rostral region of the oviduct, contains the ostium (in the portion next to the ovary), which is responsible for receiving the oocyte released upon ovulation.



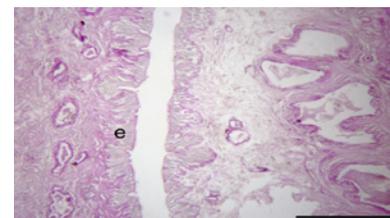
**Figure 1:** Photomicrograph of the infundibulum of *P. geoffroanus*. Mucous layer with epithelium (e) and lamina propria (l), vascularized (v) and serous layer (s). HE. 100x.



**Figure 2:** Photomicrograph of the infundibulum of *P. geoffroanus* showing simple cylindrical epithelium with ciliated and non-ciliated cells (e). HE. 100x.



**Figure 3:** Photomicrograph of the infundibulum of *P. geoffroanus* showing the negative reaction of the epithelium for acid glycoconjugates (GCs) (e). AB. 100x.



**Figure 4:** Photomicrograph of the infundibulum of *P. geoffroanus* showing the negative reaction of the epithelium for neutral glycoconjugates (GCs) (e). PAS. 100x.

| Regions      |            | Histochemical Stains |     |                  |
|--------------|------------|----------------------|-----|------------------|
|              |            | AB                   | PAS | XYLIDENE PONCEAU |
| Infundibulum | Epithelium | -                    | -   | ±                |
|              | Glands     | -                    | ±   | +                |
| Isthmus      | Epithelium | +                    | +   | -                |
|              | Glands     | -                    | -   | +                |
| Uterus       | Epithelium | -                    | -   | ±                |
|              | Glands     | ±                    | ±   | +                |
| Vagina       | Epithelium | ±                    | ±   | ±                |
|              | Gland      | +                    | +   | ±                |
|              | Epithelium |                      |     |                  |

**Table 1:** Histochemical reactions of epithelium and glands from different regions of the oviduct of *P. geoffroanus*.

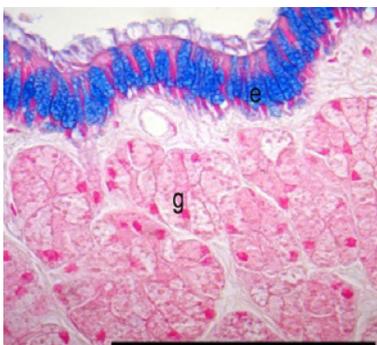
Infundibulum histology has shown a thin mucus layer (Figure 1), from which small sulci are formed, lined by a single cylindrical epithelium containing ciliated and non-ciliated cells (Figure 2). These epithelial cells show no production of acid and neutral GCs because they react negatively with AB (pH 2.5) and PAS (Figures 3,4 and Table 1). Directly below the epithelial cells lies an aglandular lamina propria formed by loose connective tissue



**Figure 5:** Photomicrograph of the uterine tube of *P. geoffroanus* showing the epithelium (e), glands (g), muscle layer (m) and serous layer (s). HE. 100x.



**Figure 6:** Photomicrograph of the uterine tube of *P. geoffroanus* showing the nuclei of epithelial cells at different heights (arrow). HE. 100x.



**Figure 7:** Photomicrograph of the uterine tube of *P. geoffroanus* showing the positive reaction of the epithelium (e) and the negative reaction of the glands (g) for acid glycoconjugates (GCs). AB. 100x.

that is richly vascularized by venules, arterioles and lymphatic vessels.

The muscle layer, which is poorly developed in this region, has bundles of smooth muscle fibers that are histologically arranged in a spiral and therefore assume a visibly longitudinal direction. The serous layer, which surrounds the muscular layer, is formed by a simple squamous epithelium lining, which is a delicate layer of connective tissue. All structures were weakly stained when analyzed by the Xylidine Ponceau method, demonstrating that the infundibulum has significant protein content (Table 1).

### Uterine tube (Magnum)

Macroscopically, the uterine tube is the largest region of the oviduct located between the infundibulum and the isthmus and typically contains shallow crypts, as well as a number of deep crypts.

The uterine-tube mucous layer, which is more developed than that

found in the infundibulum (Figure 5), is protected by a pseudostratified columnar epithelium composed of mucous-secreting cells and a few hair cells. These epithelial cells have an ovoid nucleus; however, the nuclei of secretory cells are located in the basal region, and the nuclei of ciliated cells are located in the apical region (Figure 6). The tube epithelium actively participates in the production of acid (Figure 7) and neutral GCs, as 146 indicated by this region's intense positive reaction to AB and PAS, respectively (Table 1). The lamina propria is composed of richly vascularized, loose connective tissue and a higher number of tubular glands that react weakly to PAS and exhibit no reaction to AB (pH 2.5) (Table 1), indicating that these glands may participate in the production of neutral GCs.

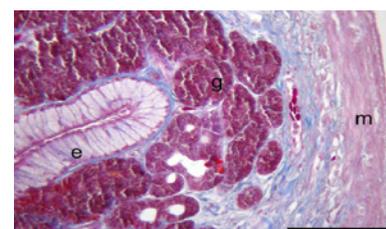
The muscle layer, located immediately below the mucosa, is formed by bundles of smooth muscle fibers arranged longitudinally (Figure 8), as found in the infundibulum. The serous layer surrounding the entire region is similar to that lining the infundibulum region.

With the exception of the epithelial cells, all structures were evenly stained when analyzed via the Xylidine Ponceau method (Table 1). This result indicates high protein content throughout the uterine tube and in its secretions, especially in the glands.

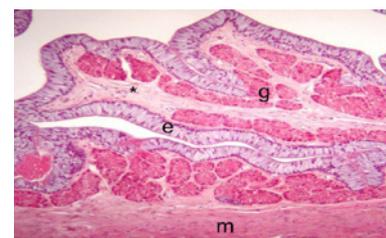
### Isthmus

Microscopic images of *P. geoffroanus* show the isthmus as a transition zone of the oviduct, that is, a junction between the uterine tube and the uterus. The mucosa typically has deeper crypts than those found in the uterine tube (Figure 9). The epithelium is pseudostratified columnar (Figure 10) with mucous-secreting tall cells and hair cells that react positively to AB (pH 2.5) and PAS (Figure 11), indicating production of acid and neutral GCs. The lamina propria of the isthmus shows a lower number of glands than the uterine tube, and these isthmus glands react negatively to AB (pH 2.5) and PAS (Figure 11) (Table 1). This lamina propria of the loose connective tissue is marked by extensive vascularization.

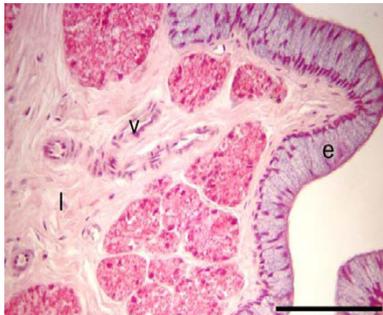
Smooth muscle fibers are visibly arranged in two directions in the



**Figure 8:** Photomicrograph of the uterine tube of *P. geoffroanus* showing the epithelium (e), glands (g) and muscle layer (m). Mallory's Trichrome. 100x.



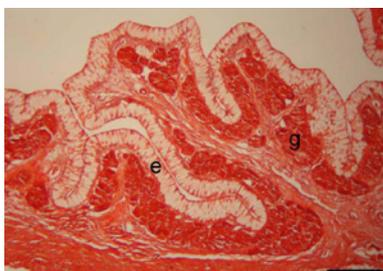
**Figure 9:** Photomicrograph of the isthmus of *P. geoffroanus* showing the epithelium (e), lamina propria of the mucosa with reduced number of glands (\*), glands (g) and muscle layer (m). HE. 100x.



**Figure 10:** Photomicrograph of the isthmus of *P. geoffroanus* showing mucous-secreting pseudostratified columnar epithelium (e) and lamina propria of the mucosa (l) vascularized (v). HE. 100x.



**Figure 11:** Photomicrograph of the isthmus of *P. geoffroanus* showing the positive reaction of the epithelium (e) and the negative reaction of the glands (g) for neutral glycoconjugates (GCs). PAS. 100x.



**Figure 12:** Photomicrograph of the isthmus of *P. geoffroanus* showing the negative reaction of the epithelium (e) for total proteins. XP. 100x.

muscle layer, an inner circular direction and an outer longitudinal direction. This layer is thicker than that of the tube but contains less of the double layer of muscle found in the uterus and vagina. The serosa of the isthmus is similar to that of the other oviduct segments.

Except for the epithelial cells (Figure 12), analysis via the Xylidine Ponceau method resulted in even staining among all structures (Table 1), as was observed in the uterine tube. This result indicates that the isthmus and its secretions have high protein content.

## Uterus

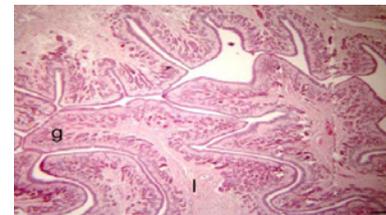
The uterus is one of the thickest oviduct regions but has an intermediate length compared to the uterine tube.

The mucosa typically has deep folds that form deep projections (Figure 13). In 184 *P. geoffroanus*, the cells forming the epithelium in this region are not as tall as those of the uterine tube and isthmus; however, as found in the uterine tube and isthmus, two cell types are

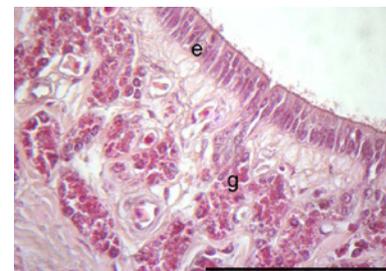
also observed, cylindrical secretory cells with basal nuclei and ciliated columnar cells with apical nuclei (Figure 14). Epithelial cells react negatively to AB (pH 2.5) (Figure 15) and PAS and therefore do not participate in the production of acid and neutral GCs (Table 1). The lamina propria of the loose connective tissue in the uterus is extensively vascularized. The presence of tubular glands has been noted in the lamina propria; these glands are henceforth termed uterine glands or shell glands. The uterine glands react negatively to the histochemical methods used to detect acid and neutral GCs (Table 1). However, cubical cells lining the duct of glands show AB- (pH 2.5) (Figure 15) and PAS-positive granules.

There is a thick layer of smooth muscle in the uterus (Figure 16), distinguishing an inner circular layer and an outer longitudinal layer, separated by a thin layer of connective tissue. The serous layer of the uterus is similar to that of the other segments.

The uterine region showed a more intense reaction in the uterine



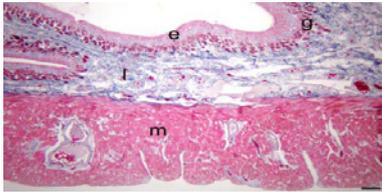
**Figure 13:** Photomicrograph of the uterus of *P. geoffroanus*. Pleated mucous layer with lamina propria of the mucosa (l) and with uterine glands (g). HE. 100x.



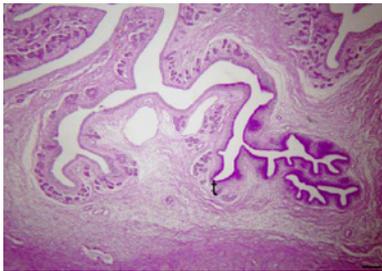
**Figure 14:** Photomicrograph of the uterus of *P. geoffroanus* showing the pseudostratified columnar epithelium with ciliated and non-ciliated cells (e) and uterine glands (g). HE. 100x.



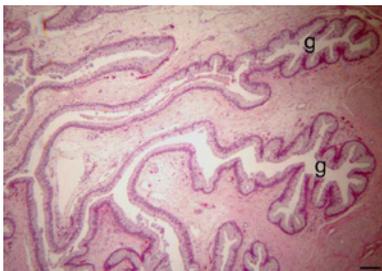
**Figure 15:** Photomicrograph of the uterus of *P. geoffroanus* showing the negative responses of the epithelium (e) and uterine glands (g) for acid glycoconjugates (GCs) and the positively reacting cells of the gland ducts (d). AB. 100x.



**Figure 16:** Photomicrograph of the uterus of *P. geoffroanus* protected by an epithelium (e), and lamina propria of the mucosa (l) with uterine glands (g) and muscle layer (m). Mallory's Trichrome. 100x.



**Figure 17:** Photomicrograph of the utero-vaginal transition of *P. geoffroanus*. PAS. 100x.



**Figure 18:** Photomicrograph of the vagina of *P. geoffroanus*. Pleated mucosal layer forming tubular glands (g). HE. 100x.

glands (shell glands) (Table 1), and cells scattered among the epithelial cells showed an even stronger staining, when submitted to the Xylidine Ponceau method. There is a gradual change from the end of the uterine segment to the vagina with similar histological characteristics in both regions (Figure 17).

## Vagina

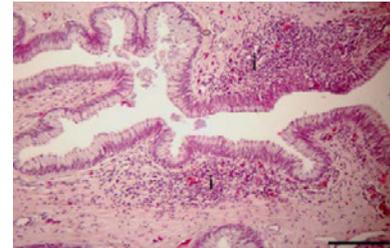
The vagina of *P. geoffroanus* is the last and thickest segment of the oviduct, which is responsible for transporting the egg through the cloaca until oviposition.

The vagina has a pleated mucosa (Figure 18) underpinned by a lamina propria of little-vascularized loose connective tissue. The vaginal mucosa is lined by a ciliated pseudostratified columnar epithelium with mucous-secreting cells that slightly reacted to the techniques for detecting acid and neutral GCs. The amount of lymphatic tissue (lymphocytic cells) found in the lamina propria of this region (Figure 19) is noteworthy. The folds of this mucous layer form compound tubular glands lined by a simple ciliated epithelium (Figure 20), and these glands flow directly into the lumen of the organ. The epithelium of these glands exhibit intense positive reactions to the AB (pH 2.5) and PAS methods (Figures 21,22

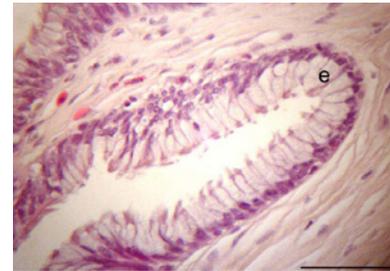
and Table 1), indicating the production of acid and neutral GCs, respectively.

The vaginal muscles are thicker (Figure 23) than the abovementioned segments, with an inner circular layer and an outer longitudinal layer that, as in other segments, are also lined by a serosa.

All layers forming the vagina of *P. geoffroanus* reacted weakly



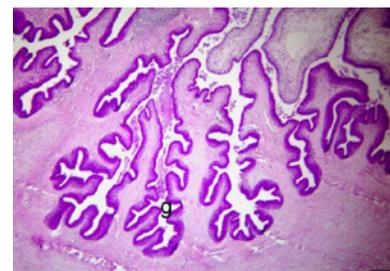
**Figure 19:** Photomicrograph of the vagina of *P. geoffroanus* showing glands lined by simple ciliated cylindrical epithelium (e). HE. 100x.



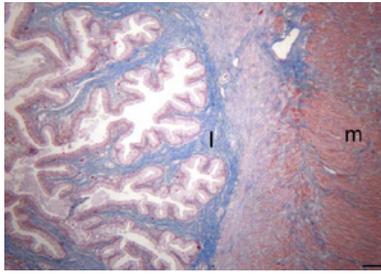
**Figure 20:** Photomicrograph of the vagina of *P. geoffroanus*. Mucosal layer with lymphocytic infiltrates (i). HE. 100x.



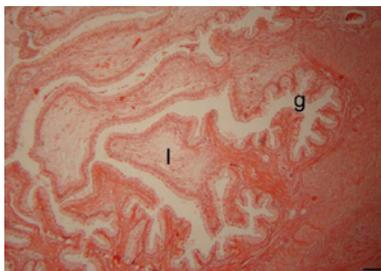
**Figure 21:** Photomicrograph of the vagina of *P. geoffroanus* showing the positive reaction of the epithelium (e) for acid glycoconjugates (GCs) in the glands. AB. 100x.



**Figure 22:** Photomicrograph of the vagina of *P. geoffroanus* showing glands protected by epithelium that tested positive for neutral glycoconjugates (GCs). PAS. 100x.



**Figure 23:** Photomicrograph of the vagina of *P. geoffroanus*. Lamina propria of the mucosa (l) and muscle layer (m). Mallory's Trichrome. 100x.



**Figure 24:** Photomicrograph of the vagina of *P. geoffroanus*. Lamina propria of the mucosa (l) and glands (g) showing weak reactions to total proteins. XP. 100x.

when analyzed via the Xylidine Ponceau method (Figure 24 and Table 1).

## Discussion

In most reptiles, the oviducts are paired structures that are dorsally situated on both sides of the body, and both oviducts are functional and separate, joining near the cloaca entry. Exceptions include certain lizards that can have vestigial oviducts, usually located on the left [13]. The Geoffroy's toadhead turtle also has two oviducts, which are located on either side of the body and are both physiologically functional.

Morphologically, the oviducts of sea and freshwater turtles can be divided into the following five distinct regions: the infundibulum, the uterine tube, the isthmus or transition zone, the uterus and the vagina [10,13-16]. This oviduct organization has also been demonstrated histologically in the *P. geoffroanus* species. However, the oviducts of other reptiles are subdivided into three or four regions, as in the oviparous lizards *Crotaphytus collaris* and *Eumeces obsoletus* [17], in the viviparous lizard *Hoplodactylus maculatus* [18], in the snakes *Diadophis punctatus* [19] and *Seminatrix pygaea* [20] and in the gecko *Hemidactylus mabouia* [21].

As found in the oviducts of other reptilian species, including *S. pygaea* [20] and *H. mabouia* [21], the *P. geoffroanus* oviduct exhibits three basic layers. From the outer surface to the lumen, these are as follows: serosa, muscle and mucosa.

In the Geoffroy's toadhead turtle, the infundibulum, which is the region responsible for receiving the oocyte from the ovary, is not subdivided in contrast to what is observed in the gopher tortoise *Gopherus polyphemus* [15] and in the lizards *C. collaris* and *E. obsoletus* [17] (which have two distinct anterior and posterior portions). Thin histological layers, composed of an undeveloped muscle layer and the lamina propria of the mucosa-lacking glands, typify the infundibulum

region [15,17,22-24]. The lamina propria of *P. geoffroanus* lies beneath a simple cylindrical epithelium composed of ciliated and non-ciliated cells. This finding is similar to observations in the serpent *D. punctatus* [19], the lizard *H. maculatus* [18] and *H. mabouia* [21]. The infundibulum reacts weakly to the Xylidine Ponceau method, indicating that it is not involved in protein synthesis, as noted in *H. mabouia* [21]. The muscle layer is thin but visible, although delimiting an inner circular layer and an outer longitudinal layer, as noted in *H. maculatus* [18], was not possible. Girling [10] has noted that there has been little discussion regarding the functions of this oviduct region, despite the detailed descriptions of the infundibulum found in the literature.

The uterine tube of *P. geoffroanus* shows the same histological layers found in the infundibulum but has thicker walls. The pseudostratified columnar epithelium that protects this region consists of mucous-secreting cylindrical tall cells and ciliated cylindrical cells. Girling [25] has argued that in turtles and crocodylians, the non-ciliated secretory cells of the uterine tube are responsible for the production of albumin. The epithelium of the tube of *P. geoffroanus* responds positively to tests that indicate the production of acid and neutral GCs (AB pH 0.4 and 2.5 and PAS), a result also found in *D. punctatus* [19], *Hemidactylus turcicus*, *Saltuarius wyberba* and *H. maculatus* [25], *S. pygaea* [20] and *H. mabouia* [21]. Those secretions may be involved in protecting and lubricating epithelial surfaces, thereby facilitating the passage of oocytes through the oviduct [20,26,27]. As found in other turtles [15,28], numerous glands populate the uterine-tube mucosa of the Geoffroy's toadhead turtle. In reptiles, these glands may be tubulo-alveolar, tubular or acinous [15,29,30]. The glands found in this region only secrete neutral GCs because they react positively only to Periodic Acid-Schiff (PAS). This result is similar to results reported by Palmer and Guillete [15], who noted a negative reaction to the histochemistry method used to detect acid GCs (AB, pH 0.4 and 2.5) and a positive reaction only for PAS. The uterine-tube glands of *P. geoffroanus* may participate in albumin secretion because they react positively to Xylidine Ponceau, indicating the presence of protein at this site. These glands have been shown to participate in albumin protein synthesis in the sea turtle *Chelydra mydas* [28] and gopher tortoise *G. polyphemus* [15]. Girling [10] argues that the uterine tube is responsible for the synthesis of albumin, an egg-white protein that surrounds the egg prior to oviposition in turtles and crocodylians. The muscular layer of the Geoffroy's toadhead turtle is arranged in only one direction in contrast to what has been observed in *H. maculatus* [18].

The isthmus is a glandular region that connects the uterine tube to the uterus. The isthmus's protein-secreting function has been confirmed via its positive reaction to Xylidine Ponceau staining. Despite this region's function and in contrast to results obtained for the uterine tube, the number of these glands in the mucosa of the isthmus is small. This finding is similar to one report regarding the freshwater turtle *Chelydra serpentina* [16] but is contrary to findings reported by other authors [10,15,25,28], who noted an aglandular isthmus. The lamina propria of the isthmus lies beneath a pseudostratified columnar epithelium containing secreting tall cells and ciliated cells (in addition to those found in the uterine tube of this species). These epithelial cells of *P. geoffroanus* participate in the secretion of acid and neutral GCs, as noted in *Tarentola m. mauritanica* [31].

The uterus is the region where eggs are retained until oviposition. The *P. geoffroanus* uterus consists of a glandular mucosa layer, which is a well-developed muscle layer and a serosa that lines the organ, as described for certain reptiles [15,28-30]. The epithelium of this region is pseudostratified columnar and is formed by ciliated and non-ciliated

cells, similar to observations in other reptiles [10,15,17-19,25,27,31-33]. The uterine epithelium reacts negatively to histochemical 300 methods that detect acid and neutral GCs, a result confirmed by Girling [18].

As found in certain oviparous lizards [17,21,25,32] and turtles [15,16,28], the uterine lamina propria is dominated by shell glands that are involved in egg-shell secretion. These glands react negatively to methods that detect acid and neutral GCs, as found in *H. maculatus*, *H. turcicus* and *H. mabouia* [18,21,25]. The shell glands found in the uterus of the Geoffroy's toadhead turtle communicate with the lumen through ducts, as found in *H. maculatus* [18]. In *P. geoffroanus*, these ducts appear to contain granules that synthesize acid and neutral GCs. The epithelium and uterine glands were both found to react positively to the histochemical method used to detect protein(s), unlike the results obtained for *H. mabouia* [21].

Below the mucosa, the uterus of this species has a thick muscle wall in which an 311 inner circular layer and an outer longitudinal layer can be differentiated. These muscle 312 layers may participate in oocyte ejection during oviposition. Several other authors have 313 reported similar results in other reptiles [15-17,19,21,25,31,32].

The vagina of *P. geoffroanus* is highly muscular and connects the entire oviduct to the urogenital sinus (cloaca). Based on the morphological characteristics of the vaginal wall, this region is not subdivided into more than one segment, similar to the findings of Girling [18] and Sánchez-Martínez [34]. However, this result differs from results obtained in different species of reptiles [21,35,36]. The authors of the latter studies have consistently reported the existence of morphological differences that justify subdivision of the vagina.

A mucous-secreting pseudostratified columnar epithelium with non-ciliated cells is found in the vagina mucous layer. This result differs from that found in the vagina of *C. serpentina* [16], which features an epithelium consisting of ciliated cells only. However, in several studies describing reptile oviduct morphology, the vaginal epithelium has been found to consist of ciliated and non-ciliated cells [10,18,20,25]. The vaginal mucosal epithelium was found to react weakly to histochemical methods that detect acid and neutral GCs, as well as methods that detect total proteins. The vaginal epithelium invades the lamina propria, forming compound tubular glands that flow into the lumen of the organ. This glandular epithelium participates in the secretion of acid and neutral GCs but, as accessed via the total protein method, does not secrete proteins. The inner circular and outer longitudinal muscle layers are the most developed across the oviduct, as noted in *H. maculatus* [18]. Fox [13] reports that the function of the vaginal canal is linked to this thickening of the muscle layer in certain lizards.

## Conclusions

The female reproductive system of *P. geoffroanus* is, in general, similar to that of other reptiles, as it is composed of a pair of oviducts that are subdivided into infundibulum, uterine tube, isthmus, uterus and vagina sections.

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