

Morphological aspects of the liver of the freshwater turtle *Phrynops geoffroanus* Schweigger, 1812 (Testudines, Chelidae)

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Abstract

The objective was the morphological characterization of the liver of *Phrynops geoffroanus*, popularly known as the freshwater turtle (cágado-de-barbicha in Portuguese), using six specimens from the Uberabinha River in Uberlândia, state of Minas Gerais, Brazil. The analyses involved anatomical and histological methods, and the microscopic aspects were described after the histological preparation. Fragments of liver were fixed in 10% formalin and absolute alcohol, embedded in paraffin, sectioned into four µm thick slices, and stained with HE, PAS, Gomori Trichrome and Reticulin. The liver of *P. geoffroanus* is a large light brown organ speckled with black spots, which represent melanin deposits. It is divided into four lobes, called the right lateral, right medial, left lateral and left medial lobes. The hepatocytes in longitudinal section are similar to double cords surrounded by winding sinusoidal capillaries, polyhedral shaped, of varying sizes, with a vacuolized aspect and with peripherally displaced nuclei. The cytoplasm is little eosinophilic and highly reactive to PAS, suggesting abundant intracytoplasmatic glycogen. The hepatic parenchyma is supported by delicate reticular fibers surrounding hepatocytes and sinusoids. Large quantities of melanomacrophages are present in the parenchyma and perisinusoidal spaces, mainly close to the portal spaces.

Keywords: chelidae, histology, morphology, reptiles, testudines.

1 Introduction

Reptiles are a diverse group of vertebrates that today are represented by turtles, crocodylians, lizards, sphenodonts and birds. They are possibly one of the most complex groups, whose order is the focus of many contemporary discussions. Testudines are characterized by the presence of a shell (carapace and plastron) joined by bone bridges and are divided according to their habit. The terrestrial ones are called tortoises, while those of marine and freshwater habits are turtles. They are edentulous, with the rostral portion of the cranium covered with a horny beak forming a grinding surface (KELLNER and SCHWANKE, 2001).

Of the world's 278 species of Testudines, approximately 20% occur in South America, represented by 20 families. Among them the family Chelidae, whose members are known popularly in Brazil as “cágados”, is the richest, with 23 species, 19 of which occur in Brazil (SOUZA, 2004). The genus *Phrynops* comprises four species, which includes *P. geoffroanus*, a small species frequently found in rivers, lakes and lagoons with slow-flowing currents and occurring from Colombia, Venezuela, and the Guianas to the extreme south of Paraguay and in the southeast, central west and northeast of Brazil (ERNEST and BARBOUR, 1989).

With regard to its food habits, Terán, Vogt and Gomez (1995) evaluated the stomach content of this species and

found 48% of seeds, fruits, and stems, 30% of fish, 10% of insects and 5% of crustaceans. Fish feed with protein levels varying from 28 to 30% of gross protein is the food most commonly used in the nutrition of these animals. The type of food may be related to the morphological aspects of the liver, probably due to the protein and fat contents in the feed, which can lead to accumulation of carbohydrates and intracytoplasmic fat. The liver tends to accumulate glycogen as a reserve for use in several situations, such as heightened sexual activity during the reproductive season and metabolic alterations at different times of the year, especially in species of cold climates where hibernation occurs (SCHAFFNER, 1998). According to Gregory (1982), significant variations of these accumulated products have been found in different species.

A few authors have described the liver of reptiles macro- and microscopically. Schaffner (1998) analyzed the liver of reptiles in general, while Henninger (1982) studied the microscopic aspects of the organ of the box turtle *Terrapene carolina* and Machado Júnior, Sousa, Carvalho et al. (2005) described the macroscopic aspects of this gland in the scorpion mud turtle *Kinosternon scorpioides*. However, the few morphological studies that have been made of

the digestive system of *P. geoffroanus* do not include a morphological description of the liver of this species.

Little is known about the morphological aspects of most South American Testudines, especially the species that occur in Brazil. Aiming to contribute to this line of research, the objective of the present work was to evaluate the liver of six specimens of *P. geoffroanus* macro- and microscopically and to describe its anatomical relationships.

2 Material and methods

The animals used in this experiment were three females and three males captured on the banks of the Uberabinha River, in the urban zone of the municipality of Uberlândia, Minas Gerais, Brazil, using hook and line, under permit number 032/2006 IBAMA-RAN. The animals' coelomic cavity was opened, completely exposing the viscera, a description was made of the anatomical relationships, and fragments were collected and fixed in 10% formalin in 0.1 M phosphate buffer and absolute alcohol.

The histological procedures were carried out in the histopathology laboratory of the Federal University of (UFU), where the fragments were processed according to the routine histological technique (BEHMER, TOLOSA and FREITAS NETO, 1976). Six slides, each containing at least two slices, were prepared from each fragment and were subjected to the staining techniques by Hematoxylin-eosin, Periodic Acid-Schiff (PAS), Gomori Trichrome and Reticulin. The slides were analyzed under an Olympus BX40 binocular microscope coupled to an Olympus OLY-200 camera and connected to a personal computer by means of a Data Translation 3153 digitizing board. The images were obtained using 10× and 40× lenses.

3 Results and discussion

Testudines can tolerate several prolonged unfavorable conditions due to their adaptive capacity, specific anatomy, and their specialized biochemistry and physiology, including conditions related to the liver (MADER, BENNETT, FUNK et al., 2006). The structural complexity of this gland has been reported in *Kinosternon scorpioides*, *Testudo horsfieldi* and *Testudo hermanni* (MACHADO JÚNIOR, SOUSA, CARVALHO et al., 2005; MARYCZ and ROGOWSKA, 2007) and now in *Phrynops geoffroanus*.

The liver of *P. geoffroanus* is wide and thin (Figure 1), with an approximately rectangular shape, filling almost the entire cranial portion of the coelomic cavity and surrounding the pancreas, duodenum and stomach (Figures 1 and 2), similarly to what has been described by Machado Júnior, Sousa, Carvalho et al. (2005) for the scorpion mud turtle *Kinosternon scorpioides*, by Marycz and Rogowska (2007) for the tortoises *T. horsfieldi* and *T. hermanni*, by Kükenthal and Matthes (1969) for the sand lizard *Lacerta agilis*, by Romer and Parsons (1985), generically for vertebrates, and by Pough, Heiser, McFarland et al. (1999) for turtles. Chou (1977) described a different morphology for *Ghyra mutilata*, characterizing its organ as robust and conical, while Fowler and Miller (1999) described this gland in ophidians as being elongated and cylindrical. According to Schaffner (1998), this is due to the fact that the body of these reptiles is elongated.

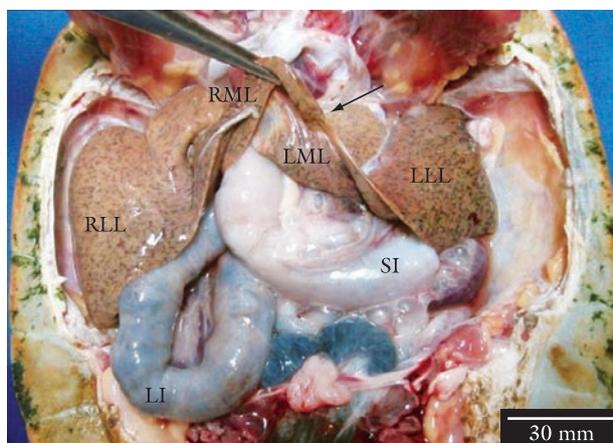


Figure 1. Photograph of the coelomic cavity (ventral view) of *Phrynops geoffroanus*, highlighting the liver. RLL - right lateral lobe; LLL - left lateral lobe; RML - right medial lobe; LML - left medial lobe; SI - small intestine; LI - large intestine; Arrow, thin aspect of the left medial lobe. Bar: 30 mm.

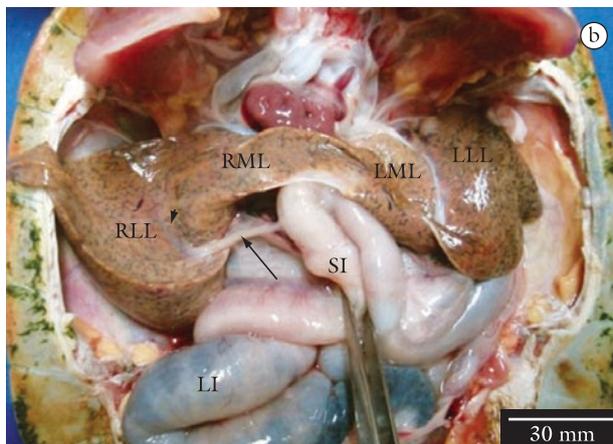


Figure 2. Photographs of the liver of *Phrynops geoffroanus*. A and B, ventral view of the coelomic cavity. RLL - right lateral lobe; RML - right medial lobe; LML - left medial lobe, LLL - left lateral lobe; SI - small intestine; LI - large intestine. Arrow, gall duct; Arrow head, gall bladder. Bar: 30 mm.

In *P. geoffroanus*, the color of the liver is pale brown mixed with black spots, which represent melanin deposits (Figures 1 and 2). Schaffner (1998) reported that the liver of reptiles varies in color from dark brown to almost black. The findings of the present work may be attributed to the greater accumulation of intracytoplasmic glycogen, which may be influenced by the diet. In such cases, the color becomes paler, especially when the blood flow diminishes. Faria (2003) states that when evaluating the amount of glycogen in hepatocytes, physiological variations should be considered in order to distinguish between the physiological and pathological status.

Due to the presence of deep fissures, the external macroscopic morphology of this organ allows for the identification of the right lateral, right medial, left lateral and left medial lobes (Figure 2). These characteristics differ from those described by Machado Júnior, Sousa, Carvalho et al. (2005) for *K. scorpoides*, whose liver has five lobes, two situated to the left and three to the right of the median plane. They also differ from the description given by Kükenthal and Matthes (1969) for *Lacerta agilis*, by Chou (1997) for *Gheyra mutilata*, by Marycz and Rogowska (2007) for the tortoises *T. horsfieldi* and *T. Hermannii*, by MacLelland (1986) for birds and by Storer, Usinger, Stebbins et al. (2000) for other species of reptiles, all of whom describe the presence of only two lobes. According to Hildebrand and Goslow (2006), the liver of vertebrates may contain several lobes arranged in various patterns that have no known functional or systematic purpose.

Schaffner (1998) and Hyman (1942) observed an unequal growth of the liver lobes of reptiles, and Marycz and Rogowska (2007) reported the same phenomenon in the tortoises *T. horsfieldi* e *T. hermannii*, whose right lobe is larger than the left one. *P. geoffroanus* also presents this difference, with the right lateral lobe the largest of the 4 lobes (Figure 2). This lobe is located in the space between the internal lateral edge of the carapace and the lateral portion of the plastron, attached to the bones of this region by a thick serous membrane.

Still with regard to the external morphology of the right lobe of this Testudines, the location of the gall bladder stands out. All the specimens analyzed showed the gall bladder immersed in the parenchyma of the right lateral lobe, from which the gall duct emerges, emptying into the duodenum. This information does not apply to the scorpion mud turtle, whose gall bladder is located between the right lateral and medial lobes (MACHADO JÚNIOR, SOUSA, CARVALHO et al., 2005). However, the differences are even greater in ophidians, whose gall bladder is set apart from the liver and positioned caudally in relation to this organ (MOSCONA, 1980; FRYE, 1991). This information indicates that there are variations in the structure and position of the gall bladder among the species of the class Reptilia.

The left lateral lobe of *P. geoffroanus* is located in the space between the edge of the carapace and the left lateral portion of the plastron, situated dorsally to the left lung. It is attached by a thick serous membrane to the bones of this region and by a thin serous membrane to the middle and final portions of the stomach, to which it is related dorsally. These characteristics are consistent with the findings of Schaffner (1998), who stated that the left lobe is connected to the stomach by the gastrohepatic ligament. Both the final portion

of the stomach and the initial portion of the duodenum are attached to the central region of the liver by a thin serous membrane. The left medial lobe is related ventrally with the heart (ventricle). A shallow gastric impression is visible in the left lateral lobe (Figure 2).

The dorsal surface of the right lateral and right medial lobes are connected to the duodenum, part of the jejunum-ileum and part of the large intestine. These lobes have a groove in which the initial portion of the duodenum is embedded. The liver is held in its anatomical position by the pressure exerted by these coelomic viscera and by the presence of ligaments that also play this role, as described for *K. scorpoides* (MACHADO JÚNIOR, SOUSA, CARVALHO et al., 2005).

The microscopic analysis indicated that the liver of *P. geoffroanus* is covered by mesothelium, underlaid by a thin layer of conjunctive tissue, the hepatic capsule, which, according to Schaffner (1998), is common to all vertebrates. According to Ross, Kaye and Pawlina (2003), this capsule contributes in the division of the parenchyma into lobules. Reticulin staining revealed that the parenchyma is supported by delicate reticular fibers surrounding hepatocytes and sinusoids, which is consistent with the findings reported by Koca, Gürcü and Balcan (2003), in their evaluation of the liver of the smooth newt (*Triturus vulgaris*).

Gardner and Oberdörster (2006) found that the structural pattern of the liver divided into lobules is not discernible in all reptile reptiles or may even be absent, as in the case of the lizards *Hemidactylus frenatus* (BRITO-GITIRANA and STORCH, 2002) and *Notophthalmus viridescens* (GOLDBLATT, HAMPTON, DIDIO et al., 1987), the broad snouted caiman *Caiman latirostris* (STARCK, CRUZ-NETO and ABE, 2007) and the fishes *Micropogon undulatus* (EURELL and HAENSLY, 1982) and *Salmo salar* (ROBERTSON and BRADLEY, 1992). Unlike the structural organization of the liver of mammals, the hepatic lobes of *P. geoffroanus* are divided into numerous lobules and consist of sinusoids and parenchymatous cells called hepatocytes (Figures 3a, b and c).

The liver of *P. geoffroanus*, as well as that of *Testudo graeca* (FERRER, ZUASTI, BALLESTA et al., 1987), differs histologically from the structural organization of some vertebrates, with hepatocytes grouped into strings or walls, forming hexagons with the portal spaces around them, starting from a central vein (GARDNER and OBERDÖRSTER, 2006). In some fishes, the hepatocytes are arranged as glands surrounded by biliary canaliculi (HAMPTON, LANTS and HILTON, 1989). In histological studies on turtles, Gardner and Oberdörster (2006) suggested a tubular arrangement of the hepatocytes with laminar strings (ELIAS and SHERRICK, 1969). These tubules have two cells in most vertebrates, including reptiles (STORCH, BRAUNBECK and WAITKUWAIT, 1989). This information is in agreement with our findings in *P. geoffroanus*, where the hepatocytes are probably arranged as cylinders which, in longitudinal section, appear as double strings surrounded by twisted sinusoidal capillaries (Figure 3), and in cross section resemble acini containing approximately two to five hepatocytes surrounding a probable central biliary canaliculus.

The hepatocytes of *P. geoffroanus* are polyhedral in shape and their sizes vary. Nuclei were observed in the central region of the cells, but most of them were shifted toward the

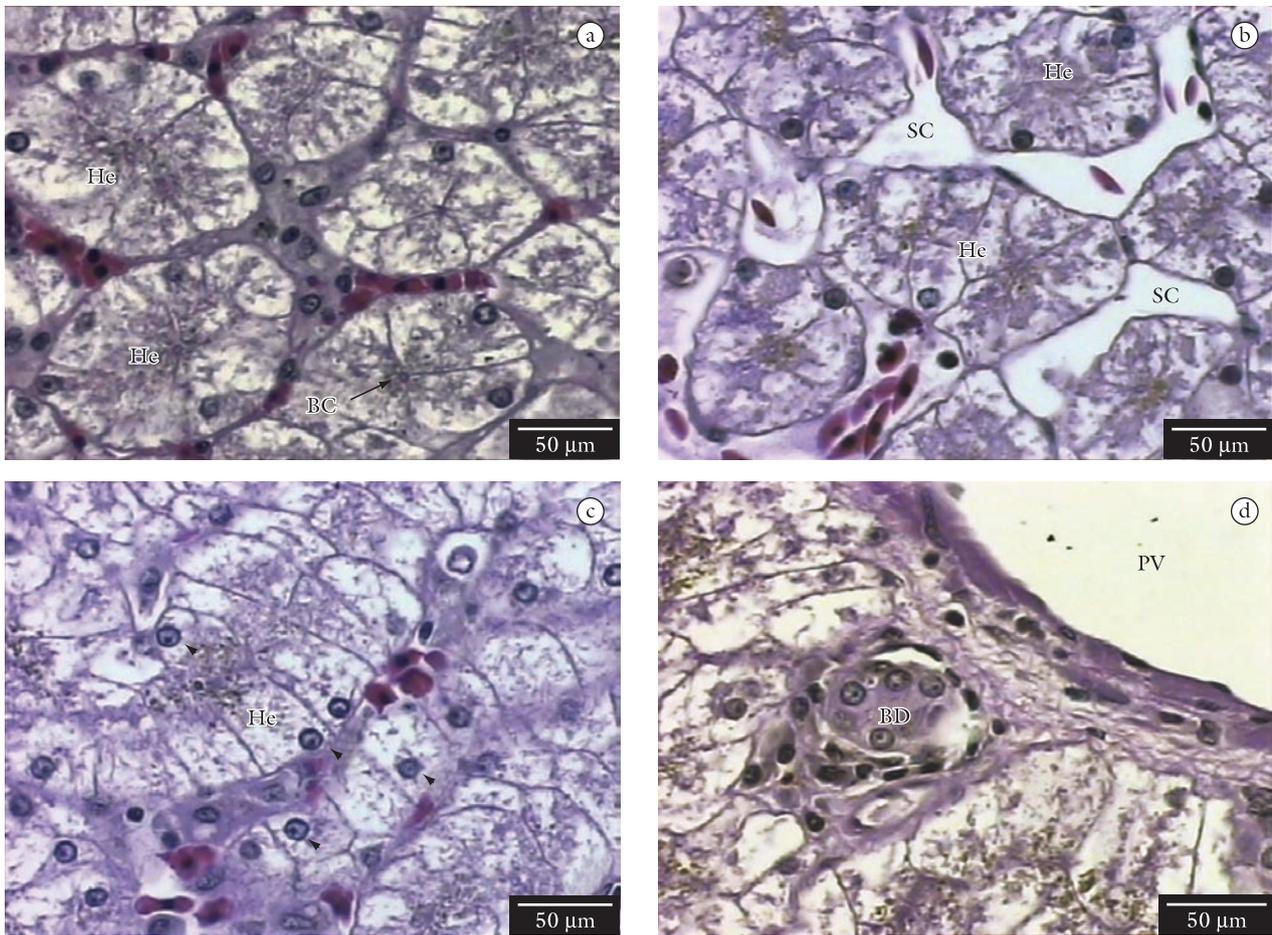


Figure 3. Photomicrographs of the liver of *Phrynops geoffroanus*. a) hepatocyte strings in cross section; Arrow → probable biliary canaliculus; b) sinusoidal capillaries; c) hepatocyte strings in longitudinal section, Arrow head ► hepatocyte nucleus; d) cuboidal epithelium of the biliary duct. He - hepatocyte; BC - biliary canaliculus; SC - sinusoidal capillaries; BD - biliary duct; PV - portal vein. Stain: Hematoxylin and eosin. Bar: 50 µm.

edge (Figure 3). This finding differs from that reported by Storch, Braunbeck and Waitkuwait (1989) for *Osteolaemus tetraspis*, who found nuclei located in the center of the cells, which are also polyhedral. The cytoplasm appeared highly vacuolated and little eosinophilic when analyzed by the hematoxylin-eosin staining technique (Figure 3), and was strongly reactive to PAS. This characteristic may be associated with the abundance of glycogen in the cytoplasm, which is not rare in reptiles.

Reticulin staining showed the hepatic parenchyma of *P. geoffroanus* supported by reticular fibers surrounding hepatocytes, blood vessels and sinusoids (Figure 4b). The same finding was reported by Koca, Gürcü and Balcan (2004) upon evaluating the liver of the smooth newt *Triturus vulgaris*, and by Petcoff, Diaz, Escalante et al. (2006) in a study of the fish *Oligosarcus jenynsii*. These fibers are characteristic of hematopoietic organs, since they are responsible for its structuring (JUNQUEIRA and CARNEIRO, 2004).

Melanomacrophages are cells with diverse functions, including the synthesis of melanin, phagocytosis and neutralization of free radicals (JOHNSON, SCHWIESOW, EKWALL et al., 1999; GUIDA, GALLONE, MAIDA et al.,

2000; SICHEL, SCALIA and CORSARO, 2002). According to Frye (1991), these cells are numerous in amphibians and reptiles, although among the latter, ophidians have very few of these cells (HACK and HELMY, 1964). In *P. geoffroanus*, all the staining techniques employed here revealed large quantities of melanomacrophages in the hepatic parenchyma (Figure 4a), similar to what Goldblatt, Hampton, Didio et al. (1987) observed in *Notophthalmus viridescens*. Several authors have studied the presence of pigments in the liver of reptiles and amphibians, including Taira and Mutoh (1981), who found a large quantity of melanin pigment in the hepatic parenchyma.

With regard to the portal tracts in *P. geoffroanus*, they were found to be supported by abundant conjunctive tissue in the interstices (Figures 4c, d). Most of the tracts contain at least one vein branch, a gall duct, and many present arteries. Gardner and Oberdörster (2006) confirm this information for other species of reptiles, similarly to what is found in mammals (BANKS, 1992; BACHA, WJJ. and BACHA, LM. 2003; SAMUELSON, 2007).

The gall bladder of *P. geoffroanus*, like that of mammals, is composed of simple columnar epithelium. Schaffner (1998) and Gardner and Oberdörster (2006) also reported

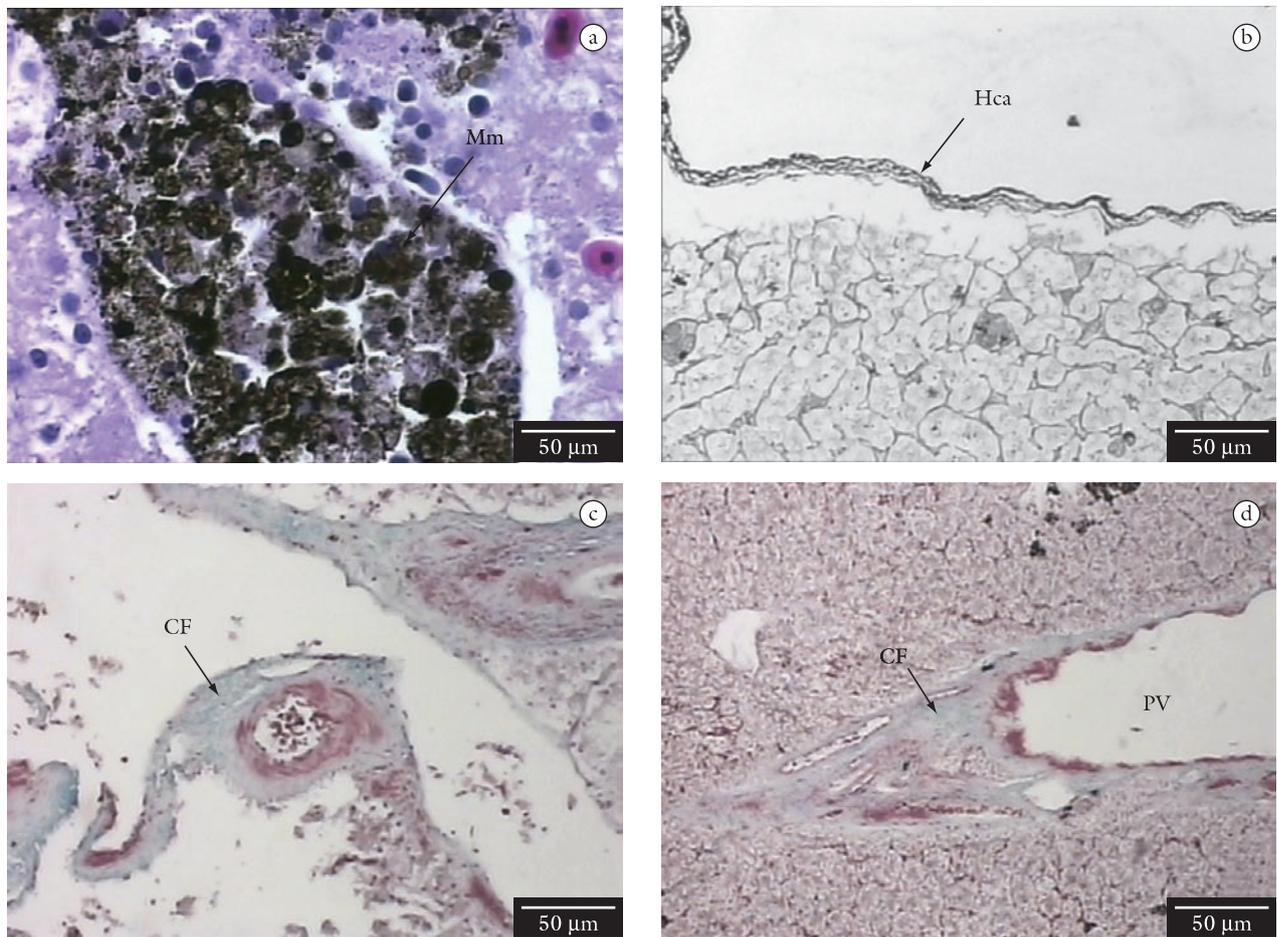


Figure 4. Photomicrographs of the liver of *Phrynops geoffroanus*. a) melanomacrophages (Mm); b) hepatic capsule (Hca); c) collagen fibers (CF); d) portal space. portal vein (PV), collagen fibers (CF). a) Hematoxylin-eosin; b) Reticulin; c and d) Gomori Trichrome. Bar: 50 µm.

the existence of pseudostratified epithelium in reptiles. Depending on its contractibility, its mucosa is pleated, as described by Schaffner (1998) for reptiles, and underlying the epithelium is a lamina proper composed of loose conjunctive tissue.

4 Conclusion

From the morphological description of the liver of *P. geoffroanus* it can be concluded that the class Reptilia shows anatomical and histological variations, as do the various species of Testudines. An aspect that stands out is the vacuolated appearance of the hepatocytes, which is similar to that observed in the glycogenoses in mammals.

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