

# Morphological aspects of the liver of the *Podocnemis expansa* (Testudines, Podocnemididae)

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## Abstract

The liver of *P. expansa* was characterized morphohistologically. To this end, twenty livers from clinically healthy male and female *Podocnemis expansa*, weighing from 2.0 to 4,5 kg, supplied by the commercial breeder Fazenda Moenda da Serra, in Araguapaz, state of Goiás, Brazil, were analyzed macro - and microscopically. The coelomatic cavity was opened and the topography of the fresh organs was examined visually. After the histological preparation, the slides were stained with Hematoxylin and Eosin (HE), Periodic Acid-Schiff (PAS), Gomori Trichrome, Reticulin and Picrosirius. The liver of *P. expansa* is a voluminous organ with an approximately rectangular shape and brown coloration, varying from light to dark shades, and is divided into a right lobe, left lobe, and a central portion. The right lobe is the largest of the three portions. The gall bladder is located in a depression in the caudal portion of the right lobe, where the gall duct begins and empties into the duodenum. Histologically, the hepatocytes are arranged in the form of double cords surrounded by winding sinusoidal capillaries. In cross section, they resemble acini containing approximately two to five hepatocytes surrounding a probable central biliary canaliculus. The hepatocytes are polyhedral or pyramidal in shape, of uniform size, with a few central nuclei and others displaced peripherally, and the cytoplasm is little eosinophilic when analyzed by the HE staining technique. The parenchyma is supported by delicate reticular fibers surrounding hepatocytes and sinusoids. The parenchyma and perisinusoidal spaces contain large quantities of melanomacrophages, mainly close to the portal spaces.

**Keywords:** histology, liver, morphology, Podocnemididae, reptiles.

## 1 Introduction

The species *Podocnemis expansa*, popularly known as the giant Amazon river turtle, or “tartaruga-da-amazônia” in Brazil, is widely distributed throughout the Amazon basin and almost all its tributaries. It is commonly found in the states of Amapá, Pará, Amazonas, Rondônia, Acre, Roraima, Tocantins, Goiás and Mato Grosso, encompassing equatorial forest and Cerrado ecosystems in the country’s Central Western region (LUZ, STRINGHINI, BATAUS et al., 2003)

According to Alho, Carvalho and Pádua (1979) this reptile is the largest freshwater Testudines of South America, reaching lengths of 75 to 107 cm, widths of 50 to 75 cm, and a live weight of about 60 kg (SMITH, 1979). It is currently considered one of the wild species most zootechnically exploited. The exoticness, flavor and quality of its meat have a higher market value than the meat of traditional domesticated animals (LUZ, STRINGHINI, BATAUS et al., 2003).

With regard to its food habits in nature Terán, Vogt and Gomez (1995) observed that the giant Amazon river turtle feeds on fruits, roots, seeds and stems of swamp plants, and suggested that vegetables account for 97% of their food. On the other hand, in captivity the animal eats every kind of food. Fish food with protein levels varying from 28% to 30% of gross protein is the food most commonly used in the nutrition of these animals (BRITO, 2007). A disorder commonly diagnosed by veterinarians working with wild animal medicine is fatty degeneration of the liver (MADER, BENNETT, FUNK et al., 2006). Thus, an understanding of the histology of the liver is essential to help clarify this disorder.

A few authors have reported on the microscopic structure of the digestive tract of Testudines. Pereira, Fonseca, Menin et al. (2005) studied the esophagus, and Gapp, Kenny

and Polak. (1985), Madrid, Ballesta, Galera et al. (1989) and Beisser, Josef Weisgram and Heinz Splechtina (1998) evaluated the tongue and esophagus. Other researchers studied the endocrine cells of the pancreas (GARCIA AYALA, LOZANO and AGULLEIRO, 1987), the intestines and the stomach (MUNIZ, MACHADO and MARQUES, 1991; IVANOVA, ROSSOL'KO and PUZYREV, 1997). Henninger (1982) focused on the microscopy of the liver of *Terrapene Carolina*, and Schaffner (1998) described the morphology of several Testudines. However, the few studies that have been made of the digestive system of *P. expansa* do not include a morphological description of the liver of this species.

To fill this gap in biological and morphological data about the liver of *P. expansa*, this study focused on a macro - and microscopic characterization of the liver this species, as well as a description of its anatomical relationships.

## 2 Material and methods

Twenty specimens of *P. expansa* were supplied for this study by the commercial breeder Fazenda Moenda da Serra, Araguapaz, state of Goiás, in the region of the middle Araguaia River where the species under study occurs naturally. The animals were clinically healthy, with an average age of three years, of both sexes (11 females and 9 males), and with a weight varying from 2.0 to 4.5 kilograms.

The animals were captured in their fattening tanks using a sweep net, weighed, individually numbered with adhesive tags on the carapace, and subjected to the following procedures: opening of the coelomic cavity by means of an incision, using a saw, of the bone bridges joining the carapace to the plastron, removing the latter to completely expose the viscera (ALVES JÚNIOR, 2006); identification of the organs of the digestive system, following the method described by Noble and Noble (1940) and Ashley (1969); description of the anatomical relationships of the liver with surrounding organs; and collection and fixation of fragments of all the lobes identified macroscopically with 3,6% formalin in 0.1 M phosphate buffer and absolute alcohol.

The fragments were processed histologically in the histopathology laboratory of the Federal University of Uberlândia (UFU), following the normal lab routine (BEHMER, TOLOSA and FREITAS NETO, 1976). Six slides were prepared from each fragment, using at least two cuts from each, which were subjected to the routine histology technique (HE), special staining (PAS, Gomori Trichrome, Reticulin) and two of these samples were stained with Picrosirius. Histological slices of approximately 80 µm stained by the latter technique were examined by confocal laser scanning microscopy (LSM 510 Meta, Zeiss) using a 10× lens and a wavelength of 543 nm.

The slides were analyzed under an Olympus BX40 binocular microscope coupled to an Olympus OLY-200 camera and connected to a computer by means of a Data Translation 3153 digitizing board. The images were obtained using 10× and 40× lenses.

## 3 Results

The liver of *Podocnemis expansa* is very voluminous and wide, representing 3.096% of the total body mass, with an approximately rectangular shape and color varying from light

to dark shades of brown. This organ fills the entire middle portion of the coelomic cavity, enveloping the pancreas, duodenum and stomach.

The external macroscopic morphology of this organ in *P. expansa* allows for the identification of the right, left and central lobes due to the presence of deep fissures (Figure 1). The right lobe is located in the space comprised between the inner edge of the carapace and the lateral portion of the plastron, attached by a thick serous membrane to the bones in this region, and its cranial portion is connected to the right cranial bony bridge. Still with respect to the external morphology of the right lobe of this turtle, a noteworthy aspect is the presence of the gall bladder (Figure 1b). All the organs analyzed here showed a cavity in the caudal portion of this lobe, from which the gall duct empties into the duodenum. An observation of the left lobe and the central lobe reveals that the former is located in the space comprised between the inner edge of the carapace and the lateral portion of the plastron in the same way as the right lobe, while the latter is long and narrow, and is joined to the two lobes. The three parts of the liver are closely connected to the stomach, especially the left lobe (Figure 1).

The static condition of this gland is ensured, among other factors, by ligaments. Both the final portion of the stomach and the initial portion of the duodenum are attached to the central lobe of the liver by a thin serous membrane. Besides its relationship to the caudal caval vein, on the dorsal portion of the right lobe this organ also suffers pressure from the surrounding viscera, as can be identified by the relationship of the caudal portion of the right lobe with the duodenum, jejunum-ileum and large intestine and the relationship of the median portion of the right lobe with the initial portion of the stomach, with emphasis on the rather shallow gastric impression in the lateral portion of the left lobe.

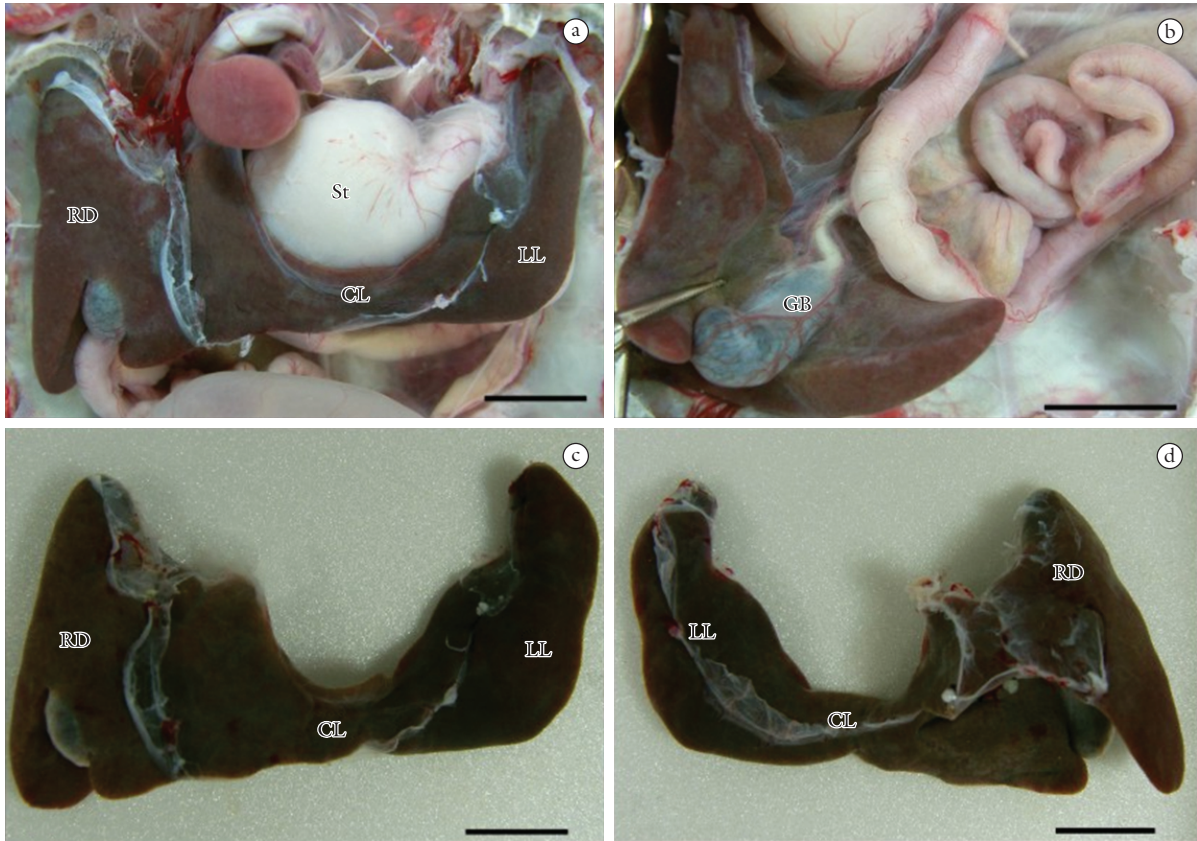
With regard to the microscopic analysis, it was found that the liver of *P. expansa* is covered by mesothelium, under which is a thin layer of conjunctive tissue, the hepatic capsule. Reticulin staining revealed that the parenchyma is supported by delicate reticular fibers surrounding the hepatocytes, vessels of the portal space and sinusoidal capillaries (Figure 4). In the species *P. expansa*, the hepatic lobes are divided into numerous lobules, which differ from those of mammals in their degree of organization, and consist of sinusoids and parenchymatous cells called hepatocytes (Figures 3a, b).

Histologically, the hepatocytes in the liver of *P. expansa* in longitudinal section resemble double strings of cells surrounded by twisted sinusoidal capillaries (Figure 2). In cross section, they resemble acini, containing approximately two to five hepatocytes surrounding a probable central biliary canaliculus (Figure 3a). Most hepatocytes are polyhedral in shape and present a wedge or pyramidal shape in histological slices, with the pyramidal shape visible in cross section and the polyhedral shape in longitudinal section. The cytoplasm appeared little eosinophilic when analyzed by the hematoxylin-eosin staining technique (Figure 3); moreover, it was reactive to PAS, indicating the presence of glycogen. All the staining techniques used here revealed large quantities of melanomacrophages in the hepatic parenchyma of *P. expansa* (Figure 3d).

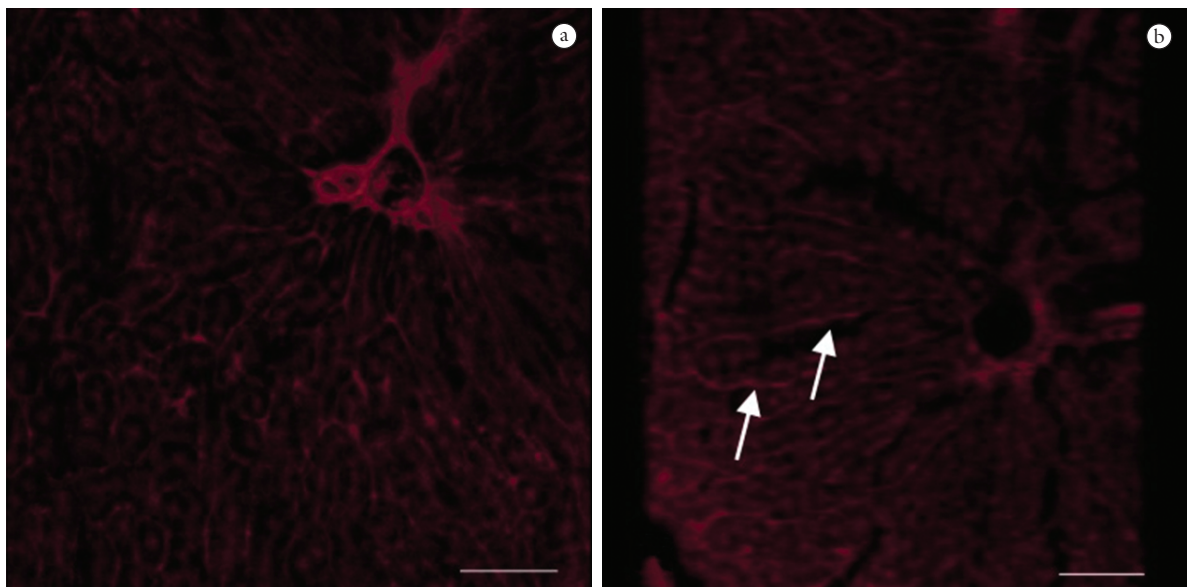
With regard to the portal tracts of *P. expansa*, they were found to be supported by conjunctive tissue in the interstices. Most of the portal spaces present a branch of a portal vein

and a gall duct, but few present arteries. A network of reticular fibers was observed between the hepatocytes and in the portal spaces. Moreover, cuboidal epithelium was found over the portal space, in the gall duct, and simple prismatic epithelium in the larger ducts.

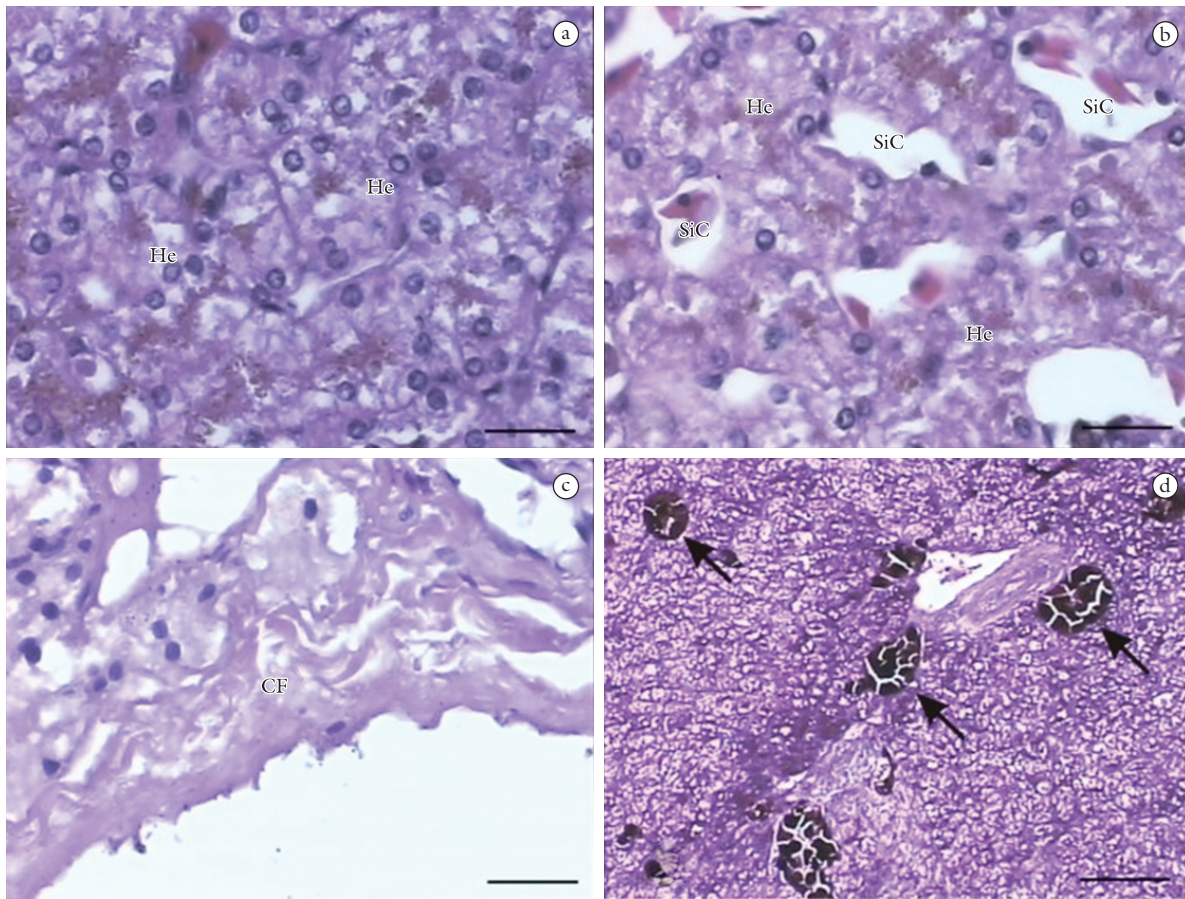
The gall bladder is composed of simple columnar epithelium. Depending on its contractibility, its mucosa is pleated. Underneath the epithelium are a lamina proper constituted of loose conjunctive tissue and an adventitious one containing abundant collagen fibers.



**Figure 1.** Photographs of the liver of *Podocnemis expansa*. a and b) ventral view of the coelomic cavity; c) ventral view of the liver outside the cavity; d) dorsal view of the liver outside the cavity. RD, right lobe; LL, left lobe; CL, central lobe, St, stomach; GB, gall bladder. Scale bar: 30 mm.



**Figure 2.** Photomicrographs of the liver of *Podocnemis expansa*. a) Portal space; b) Organization of the tubules. Arrows, collagen fibers surrounding the hepatocytes. Confocal, Picrosirius. Scale bar: 200  $\mu$ m.



**Figure 3.** Photomicrographs of the liver of *Podocnemis expansa*. a) hepatocyte strings (He) in cross section; b) sinusoidal capillaries (SiC); c) collagen fibers (CF) of the hepatic capsule; d) melanomacrophages (Arrows). Stain: Hematoxylin and eosin, a, b and c scale bar: 50 µm; d scale bar: 12.5 µm.

#### 4 Discussion

According to Gardner and Oberdörster (2006), the morphology of the liver of reptiles can vary greatly among the species of this class. The organ usually grows until it fills the entire available space in the abdominal region between the heart and the stomach. In Testudines the liver is wide, situated ventrally, and acquires its shape by spreading from one lateral margin to the other under the lungs, usually representing 2 to 5% of the total body mass (SCHAFFNER, 1998). This information can be confirmed in *P. expansa*, in which this organ is very voluminous and wide, representing 3.096% of the total body mass, with an approximately rectangular shape and color varying from light to dark shades of brown. The liver fills the entire middle portion of the coelomic cavity, surrounding the pancreas, duodenum and stomach.

A similar characteristic was described by Machado Júnior, Sousa, Carvalho et al. (2005) for the scorpion mud turtle *Kinosternon scorpioides*, by Kükental and Matthes (1969) for the sand lizard *Lacerta agilis*, by Romer and Parsons (1985) for vertebrates in general, and by Pough, Heiser and McFarland (1999) for turtles. However, Chou (1977) did not find this characteristic in *Gbeya mutilata*, but reported that the liver of this animal is robust and conical, occupying the anterior half of the abdominal cavity. The most marked differences are found in the ophidians and in some Squamates,

whose liver has an elongated and elliptical shape (FOWLER and MILLER, 1999). According to Schaffner (1998), this is due to the fact that these reptiles have long bodies.

The external macroscopic morphology of this organ in *P. expansa* allows for the identification of the right, left and central lobes due to the presence of deep fissures (Figure 1). These observations differ from those described by Machado Júnior, Sousa, Carvalho et al. (2005) for *K. scorpioides*, who report the presence of five lobes in the liver of these animals, two situated to the left and three to the right of the median plane. They also differ from those described by Kükental and Matthes (1969) for *Lacerta agilis*, by Chou (1977) for *Gbeya mutilata*, by MacLelland (1986) for birds, and by Storer, Usinger, Stebbins et al. (2000) for other species of reptiles, all of whom report the presence of only two hepatic lobes in these animals. According to Hildebrand and Goslow (2006), the liver of vertebrates can contain various lobes arranged in different patterns that have no known functional or systematic purpose.

Similarly, Schaffner (1998) identified different sized lobes in the liver of reptiles, reporting that the right lobe is the larger one. In *P. expansa*, this is located in the space comprised between the inner edge of the carapace and the lateral portion of the plastron, attached by a thick serous membrane to the bones in this region, and its cranial portion is connected to the right cranial bony bridge. Still with respect to the external morphology of the right lobe of this

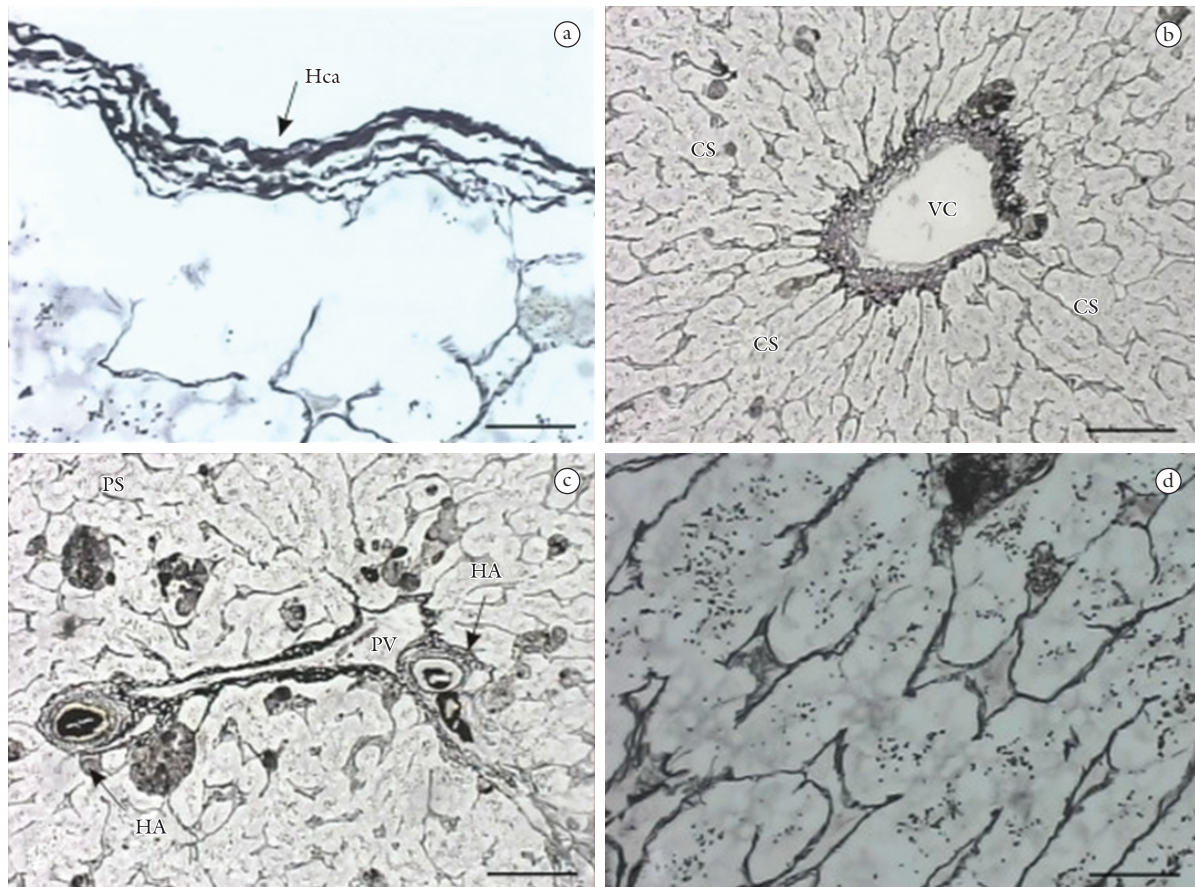
turtle, one thing that stands out is the presence of the gall bladder (Figure 1b). All the organs analyzed here showed a cavity in the caudal portion of this lobe, from which the gall duct empties into the duodenum, as Pritchard (1979) found in many reptiles, as well as in mammals. This information differs partially from what has been described for the scorpion mud turtle, whose gall bladder is located between the lateral and medial right lobes (MACHADO JÚNIOR, SOUSA, CARVALHO et al., 2005). However, ophidians show an even more notable divergence, in that the gall bladder is separate from the liver and positioned caudally in relation to it (MOSCONA, 1980). This information allows one to state that there are variations in the structure and position of the gall bladder among the species of the class Reptilia.

An observation of the left lobe and the central lobe reveals that the former is located in the space comprised between the inner edge of the carapace and the lateral portion of the plastron in the same way as the right lobe, while the latter is long and narrow, and is joined to the two lobes. The three parts of the liver are closely connected to the stomach, especially the left lobe (Figure 1). This morphology differs from that described by Storer, Usinger, Stebbins et al. (2000) for the majority of reptiles, since they reported that the liver is located cranially in relation to the stomach.

The static condition of this gland is ensured, among other factors, by means of ligaments. Both the final portion of the stomach and the initial portion of the duodenum are attached to the central lobe of the liver by a thin serous

membrane. Besides its relationship to the caudal caval vein, on the dorsal portion of the right lobe this organ also suffers pressure from the surrounding viscera, as can be identified by the relationship of the caudal portion of the right lobe with the duodenum, jejunum-ileum and large intestine and the relationship of the median portion of the right lobe with the initial portion of the stomach, with emphasis on the rather shallow gastric impression in the lateral portion of the left lobe. In ophidians, these aspects differ from those described for *P. expansa*, since the liver of snakes is positioned next to the right lung or retroperitoneal along the posterior wall of the body (DAVIS, 1981; KNOBEL, SCHOEMAN, VAN et al., 1976).

With regard to the microscopic analysis, it was found that the liver of *P. expansa* is covered by mesothelium, under which is a thin layer of conjunctive tissue, the hepatic capsule, which, according to Schaffner (1998), is common to all the vertebrates (Figures 3c and 4a). According to Ross, Kaye and Pawlina (2003), this capsule contributes to divide the parenchyma into lobules. Beresford (1993) also reported fibrous trabeculae in the hepatic parenchyma of *Alligator mississippiensis*, which strengthen this organ against sudden movements. Reticulin staining revealed that the parenchyma is supported by delicate reticular fibers surrounding the hepatocytes, vessels of the portal space and sinusoidal capillaries (Figure 4), corroborating the findings of Koca, Gúrcú and Balcan (2004) in the liver of the smooth newt (*Triturus vulgaris*).



**Figure 4.** Photomicrographs of the liver of *Podocnemis expansa*. a) reticular fibers of the hepatic capsule; b) central vein; c) portal space; d) reticular fibers in tubules and sinusoids. PV, portal vein; HA, hepatic artery; PS, portal space; HCa, hepatic capsule; HS, hepatocyte strings. Reticulin. a and d scale bar: 50  $\mu$ m; b and c scale bar: 12.5  $\mu$ m.

For Gardner and Oberdörster (2006), the structural pattern of the liver divided into lobules is not discernible in all reptile species, or may even be completely 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 fish species *Micropogon undulatus* (EURELL and HAENSLY, 1982) and *Salmo salar* (ROBERTSON and BRADLEY, 1992). In the species *P. expansa*, the hepatic lobes are divided into numerous lobules, which differ from those of mammals in their degree of organization, and consist of sinusoids and parenchymatous cells called hepatocytes (Figures 3a, b).

Histologically, the liver of *P. expansa*, as well as that of *Testudo graeca* (FERRER, ZUASTI, BALLESTA et al., 1987), differs from the structural organization of this organ in mammals, where the hepatocytes are arranged in strings or walls, forming hexagons with the portal spaces around them, starting from a central vein (GARDNER and OBERDÖRSTER, 2006). In some fishes, for example, the hepatocytes are arranged as glands surrounded by biliary canaliculi (HAMPTON, LANTS, HILTON et al., 1989). In histological studies of turtles, Gardner and Oberdörster (2006) suggest a tubular arrangement of hepatocytes into laminar strings (ELIAS and SHERRICK, 1969). These tubules have two cells in the majority of vertebrates, including reptiles (STORCH, BRAUNBECK and WAITKUWAIT, 1989) and domestic fowl (BACHA and BACHA, 2003). This information is consistent with the findings in *P. expansa*, in which the hepatocytes in longitudinal section resemble double strings of cells surrounded by twisted sinusoidal capillaries (Figure 2). In cross section, they resemble acini, containing approximately two to five hepatocytes surrounding a probable central biliary canaliculus (Figure 3a).

According to Schaffner (1998), the arrangement of the cells of the liver, in most reptiles, appears to be in an intermediate evolutionary position between the organization of the liver of fishes and mammals, with the formation of some glands and tubules. This information differs from that found in *P. expansa*.

Most of the details of the structure of hepatocytes have been obtained from studies of the liver of mammals (JONES and AGGELER, 1995). The available information indicates that reptiles and other vertebrates share many similar traits. According to Henninger (1982), most hepatocytes are polyhedral in shape and appear in wedge or pyramidal form in histological slices. These characteristics have been found in *P. expansa*, as well as by Storch, Braunbeck and Waitkuwait (1989) in *Osteolaemus tetraspis*, with the pyramidal shape visible in cross section and the polyhedral shape in longitudinal section. On the other hand, Ferrer, Zuasti, Ballesta et al. (1987) describe the hepatocytes in *Testudo graeca* as having only a polygonal shape, without giving further details of their structure.

The cytoplasm appeared little eosinophilic when analyzed by the hematoxylin-eosin staining technique (Figure 3); moreover, it was reactive to PAS, indicating the presence of glycogen. The same thing was observed by Ferrer, Zuasti, Ballesta et al. (1987) for *Testudo graeca* and by Goldblatt,

Hampton, Didio et al. (1987) for *Notophthalmus viridescens*. According to Da Silva and Migliorini (1990), this is a characteristic commonly found in healthy individuals, since the stock of hepatic glycogen represents the highest energetic stock, which can be used during a long fast, as has been observed in *Phrynosoma hilarii*.

All the staining techniques used here revealed large quantities of melanomacrophages in the hepatic parenchyma of *P. expansa* (Figure 3d). The function of these cells includes, among other things, the synthesis of melanin and neutralization of free radicals (JOHNSON, SCHWIESOW, EKWALL et al., 1999; GUIDA, GALLONE, MAIDA et al., 2000; SICHEL, SCALIA and CORSARO, 2002). According to Christiansen, Grzybowski and Kodama (1996), in turtles these cells increase with age. Melanomacrophages have been found in the liver, spleen and kidneys of fishes (AGIUS and ROBERTS, 2003), anuran (CICERO, SCUITO, CHILLEMI et al., 1982; SICHEL, SCALIA and CORSARO, 2002) and also aggregated in the liver and spleen of reptiles (SCALIA, GEREMIA, CORSARO et al., 1988; CHRISTIANSEN, GRZYBOWSKI and KODAMA, 1996). The same finding was confirmed in the histological slices of liver of *P. expansa*, similar to that reported by Goldblatt, Hampton, Didio et al. (1987) in their analysis of the liver of *Notophthalmus viridescens*.

With regard to the portal tracts of *P. expansa*, they were found to be supported by conjunctive tissue in the interstices. Most of the portal spaces present a branch of a portal vein and a gall duct, but few present arteries. In a histological study of the liver of *Terrapene carolina*, Henninger (1982) did not describe the presence of the hepatic artery in the portal tract. This information differs from that described by Gardner and Oberdörster (2006) for other species of reptiles, similarly to what is found in mammals (BACHA and BACHA, 2003; JUNQUEIRA and CARNEIRO, 2004). In both *P. expansa* and *Testudo graeca*, a network of reticular fibers was observed between the hepatocytes and in the portal spaces (FERRER, ZUASTI, BALLESTA et al., 1987). Moreover, cuboidal epithelium was found over the portal space, in the gall duct, similarly to what occurs in *Notophthalmus viridescens* (GOLDBLATT, HAMPTON, DIDIO et al., 1987), and simple prismatic epithelium was found in the larger ducts.

The gall bladder of *P. expansa*, as that of mammals, is composed of simple columnar epithelium. Gardner and Oberdörster (2006) and Schaffner (1998) also reported the existence of pseudostratified epithelium in reptiles. Depending on its contractibility, its mucosa is pleated, as described by Schaffner (1998) for reptiles. Underneath the epithelium are a lamina propria constituted of loose conjunctive tissue and an adventitious one containing abundant collagen fibers.

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