

# Histological study of the liver of the lizard *Tropidurus torquatus* Wied 1820, (Squamata: Tropiduridae)

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## Abstract

The objective of this study was to characterize macroscopically and microscopically the liver of the collared lizard *Tropidurus torquatus*, using two adult females and two adult males, captured on the banks of Marambaia Isle in Sepetiba Bay in the state of Rio de Janeiro. Fragments of liver were fixed in Bouin's liquid and submitted to routine histological techniques. Sections were cut by a rotary microtome to 5 mm thickness and stained by with HE, PAS, Gomori Trichrome and Gomori's reticulin impregnation. The liver this lizard is a large, speckled dark brown organ composed of a right and left lobe. The gallbladder is located on the visceral surface of the right lobe. The lobules are polyhedral in shape and separated by a thin layer of interlobular connective tissue, forming trabeculae. These contain bile ducts, branches of portal vein and of hepatic artery. The hepatocytes are also polyhedral and have varied sizes. The majority of these cells have nuclei offset toward the periphery, and the cytoplasm is highly vacuolated and was not well evidenced by the histological techniques utilized. The hepatic parenchyma is supported by delicate reticular fibers surrounding hepatocytes and sinusoids. Large quantities of melano-macrophage centers are present in the parenchyma.

**Keywords:** tropiduridae, lizard, liver, histology, reptiles.

## 1 Introduction

The Reptilia class is a prominent part of all terrestrial assemblage, containing 9,084 species by the latest count (UETZ and HALLERMANN, 2010). Brazil stands in second place in the world in number of reptile species (BÉRNILS, 2010). Reptiles are represented by four orders: Crocodylia, Rhynchocephalia; Squamata and Testudinata (VITT, PIANKA, COOPER-JUNIOR et al., 2003). The Squamata order includes lizards, land animals with well-defined eating habits and that are restricted by their phylogeny (VITT, PIANKA, COOPER-JUNIOR et al., 2003).

Reptiles are very important for maintenance of ecosystems. For this reason, many papers have been published on their geographic and population distribution and ecological and behavioral aspects. However, few studies have been published on the morphological aspects of these animals. These factors are important for their correct classification and comparison against other phylogenetically related species and to establish their interaction with the biome where they live.

The species of the *Tropidurus* genus are considered to be omnivores (VAN SLUYS, 1995) and occur in open areas of South America and the in the Galapagos Islands. The species *Tropidurus torquatus* is dark colored with light spots. Because it is a very common lizard species in neotropical areas, there have been several studies of its geographic and population distribution and ecological and behavioral aspects. However, there are few studies this species' morphological aspects.

The liver has multiple and complex functions. It is the site of numerous metabolic processes, because it receives all the material absorbed by the intestines, except for a

certain quantity of lipids transported by the lymph nodes (SAMUELSON, 2007). The organ is one of the most important in the organism, because it metabolizes, stores, synthesizes and eliminates the substances absorbed. It also produces bile, an exocrine secretion of the hepatic cells, which plays a key role in the digestion and absorption of fats (POUGH, HEISER and McFARLAND, 1999).

In reptiles, the liver is the largest extrinsic digestive gland and is the site of initial processing of materials absorbed by intestinal capillaries and transported via tributaries of the hepatic portal vein (SCHAFFNER, 1998). This visceral organ is large and singular and can have various shapes depending on the visceral cavity. In snakes and some lizards it is long and thin while it is thick and more compact in some other reptile species. The hepatic cells of lizards are similar to those of other vertebrates, except for the melanomacrophages, which are absent in birds and mammals (CICERO, SCUITO, CHILEMI et al., 1982; SICHEL, SCALIA and CORSARO, 2002; AGIUS and ROBERTS, 2003).

The liver takes part, among others functions, in the production and storage of glycogen, which serves as an energy reserve for use in various situations, such as sexual activity during the reproductive period and metabolic changes in different periods of the year, especially in species native to cold climates where hibernation occurs (SCHAFFNER, 1998; MARYCZ, KLEĆKOWSKA-NAWROT, MAKSYMOWICZ et al., 2009).

Knowledge of reptiles' liver morphology is a valuable source of information for clinicians. It is fundamental for

the proper diagnosis of reptile diseases. Schaffner (1998) analyzed the livers of reptiles in general, but few studies have examined the liver of *T. torquatus*. Therefore, the purpose of this study was to characterize the liver of this species macroscopically and microscopically, to shed light on the morphological aspects of this class of reptile.

## 2 Materials and methods

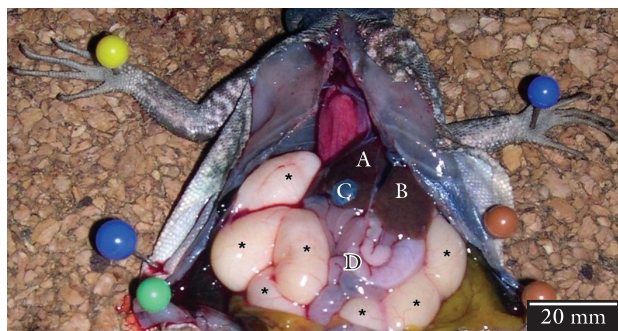
Two male and two female adults were used in this experiment, captured on the banks of Marambaia Isle (23° 04' S and 43° 53' W), located in Sepetiba Bay, Rio de Janeiro state, Brazil. After capture the specimens were taken to the Histology and Embryology Laboratory of Rio de Janeiro Federal Rural University (UFRRJ), where they were sacrificed by an overdose of 0.5% lidocaine, according to the recommendations for reptile sacrifice contained in Resolution 714 of 2002 issued by the Federal Council of Veterinary Medicine. The four lizards were measured (rostro-anal length, in mm), weighed (digital scale, precision = 0.1 g) and sexed. This research was approved by the research ethics committee (COMEP) of UFRRJ (process number 088/2010).

The animals' coelomic cavity was opened, completely exposing the viscera (Figure 1), and liver fragments were removed and immediately fixed in Bouin's liquid (DI FIORI, 1975) for six hours and submitted to routine histological techniques. Histological sections were cut by a rotary microtome to 5 mm thickness. Slides, each containing at least two slices, were prepared from each fragment and were subjected to staining by hematoxylin-eosin (HE), periodic acid-Schiff (PAS) + hematoxylin, Gomori's trichrome (GT) and Gomori's reticulin impregnation (GR). The slides were analyzed under an Olympus BX41 binocular microscope coupled to a Nikon Coolpix 4300 digital camera.

After the experiments, the specimens were deposited in the Herpetology Collection of UFRRJ, registered as from Ilha da Marambaia, Mangaratiba, Rio de Janeiro, Brazil (RU 6883; RU 6884; RU 6885 and RU 6886).

## 3 Results and discussion

The liver of *T. torquatus* is a large dark brown organ with lighter speckles, composed of a right and left lobe, located forward of the stomach. The rostro-anal length in the two females measured 18.0 and 18.5 cm and in the



**Figure 1.** Topography of intestinal organs. Right lobe of the liver (A), left lobe of the liver (B), gallbladder (C), intestine (D). Note the presence of elliptical eggs in the oviducts (\*). Bar: 20 mm.

two males 15.5 and 18.5 cm. The females weighed 16.36 and 17.34 g and the males weighed 14.87 and 22.81 g. The weight difference between the two males was because one of the animals had lost part of its tail and the regenerated part was still growing.

The microscopic analysis revealed that the liver is covered by a thin layer of connective tissue forming the hepatic capsule (Figure 2a), which according to Schaffner (1998) is common to all vertebrates. According to Ross, Kaye and Pawllna (2003), this capsule contributes to the division of the parenchyma into structural units, called hepatic lobules. These are polygonal in shape and separated by the thin layer of connective tissue, but the trabecules that have a greater quantity of this tissue allow visualization of the interlobular bile ducts, branches of portal vein and of hepatic artery (Figure 2b). The central point of the liver is the hilus, through which the portal vein and the liver artery pass. We also noted leucocytic infiltrates in the hepatic parenchyma (Figure 2c), suggesting the possible occurrence of an inflammatory process.

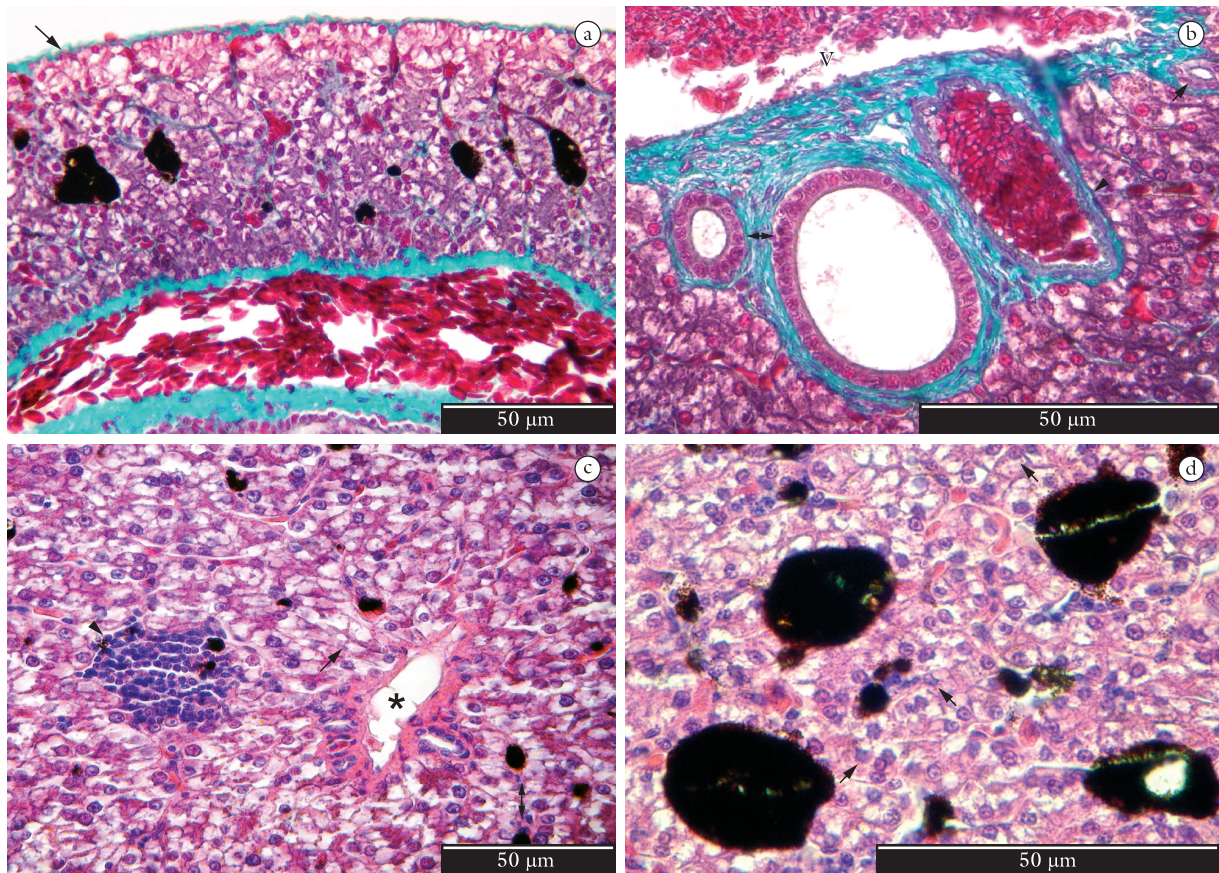
### 3.1 Hepatocytes

The hepatocytes (Figure 2d) are separated from the sinusoids by a narrow subendothelial space called the Disse space. It permits the flow of molecules between the sinusoids and hepatocytes and promotes the structural integrity of the hepatic parenchyma (SASSE, SPORNITZ and MALY, 1992). The sinusoids are capillaries covered by endothelial cells and macrophages, which in the liver are called Kupffer cells (SAMUELSON, 2007).

The hepatocytes of *T. torquatus* are polyhedral and come in varied sizes. We observed some nuclei located in the central region of these cells, but most where displaced toward the periphery (Figure 2d). This same observation was reported for *P. geoffroanus* (MOURA, SANTOS, BELLETI et al., 2009) but is different from that observed in *Osteolaemus tetraspis* (STORCH, BRAUNBECK and WAITKUWAIT, 1989), in which the nuclei, also polyhedral, are located in the center of the cells.

In the liver of *T. torquatus* the hepatocytes are arranged in cords along the sinusoids but the radial arrangement is only visible in those that are near the centrolobular vein (Figures 2c and 3a), as is also observed in mammals (SAMUELSON, 2007). In *Testudo graeca* (FERRER, ZUASTI, BALLESTA et al., 1987) and *P. geoffroanus* (MOURA, SANTOS, BELLETI et al., 2009), the hepatocytes are also grouped in cords, but these form hexagons, starting at the centrolobular vein, with portal spaces on their periphery (GARDNER and OBERDÖRSTER, 2006). In some fish species the hepatocytes are less often arranged in cords and instead form glands (HAMPTON, LANTS and HILTON, 1989).

The cytoplasm of the hepatocytes of *T. torquatus* appeared highly vacuolated and were only lightly stained by HE (Figure 3a), and reacted weakly to PAS (Figure 3b). These findings differ from those observed in *Trachomys scripta elegans* (MARYCZ, KLEĆKOWSKA-NAWROT, MAKSYMOWICZ et al., 2009), in which the results indicated an abundance of glycogen in the cytoplasm. According to Da Silva and Migliorini (1990), this is a characteristic commonly found in healthy individuals, since



**Figure 2.** Photomicrographs of the liver of *T. torquatus*. a) Connective tissue capsule (arrow). (GT). b) Hepatic lobes with interlobular space containing bile ducts (arrows) and branches of portal vein (V) and of hepatic artery (arrowhead). (HE). c) Hepatocytes arranged radially in relation to the centrolobular vein (\*), leukocyte infiltrate (arrowhead) and melano-macrophage centers (double arrow) (HE). d) Hepatocytes with nuclei displaced toward the periphery (arrows). (HE). Bar: 50 µm.

the stock of hepatic glycogen is the main reserve energy source, drawn on during prolonged fasting.

The cytoplasmic vacuolations observed in the *T. torquatus* specimens can be related to the animals' nutritional state, because they were not fed between the time of capture and sacrifice in the laboratory and also because no method was used during the histological preparation to preserve the integrity of the intracytoplasmic inclusions.

Impregnation with Gomori's reticulin revealed that the parenchyma is supported by delicate reticular fibers that surround the hepatocytes and sinusoids (Figure 3c). This result agrees with the findings by Koca, Gúrcú and Balcan (2003), analyzing the liver of the salamander *Triturus vulgaris*, by Petcoff, Diaz, Escalante et al. (2006), in a study of the fish *Oligosarcus jenynsii*, and by Moura, Santos, Belletti et al. (2009), analyzing the liver of the turtles *Phrynosops geoffroanus* and *Podocnemis geoffroanus*.

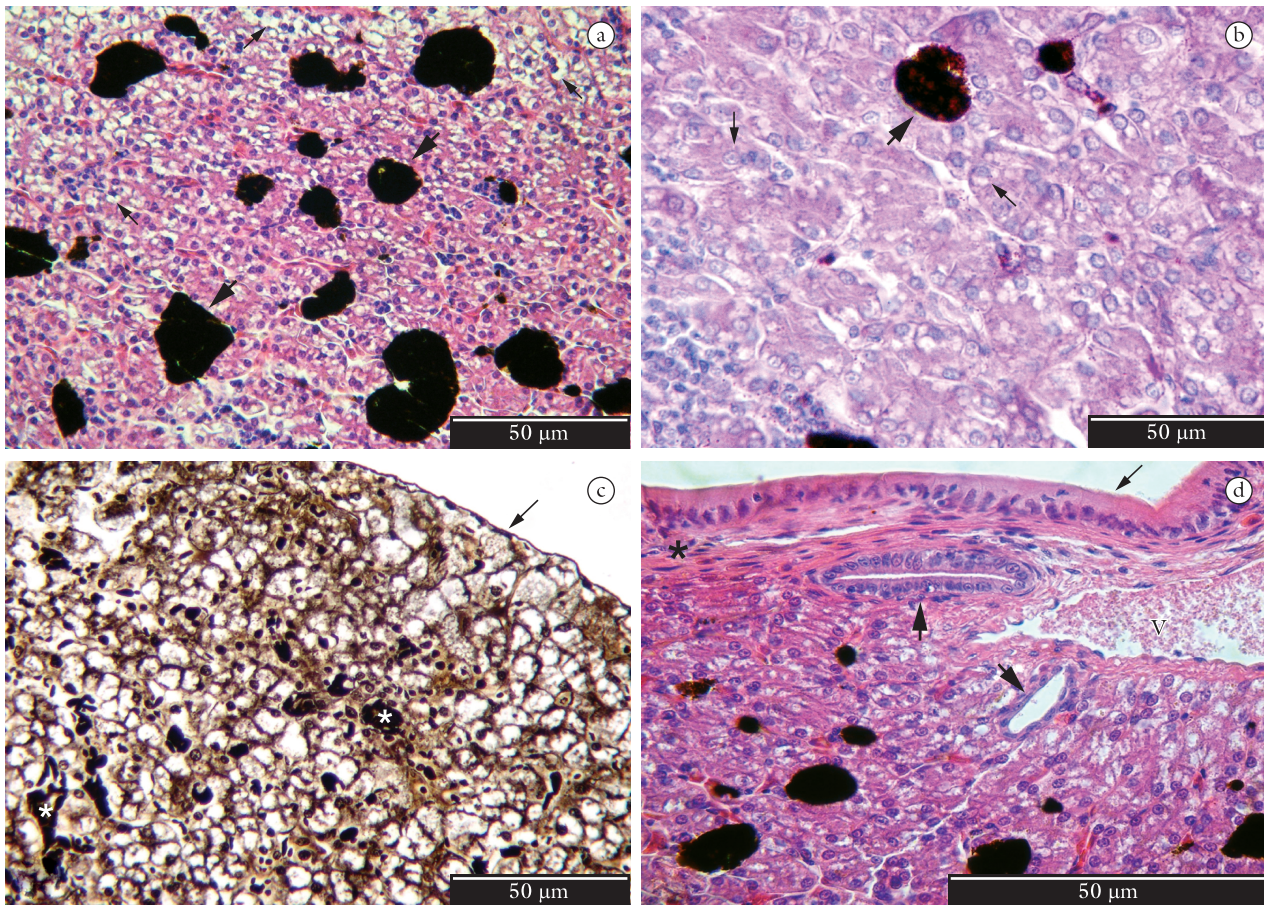
### 3.2 Melano-Macrophage centers

In the hepatic parenchyma of *T. torquatus* all the staining techniques utilized allowed observation of a large quantity of melano-macrophage centers (MMCs), as indicated in the figures already presented. These, also known as macrophage aggregates, are distinctive groupings of pigment-containing cells called melano-macrophages (MMs). They are contained

in the tissues of amphibians, reptiles and some fish, normally in the liver (AGIUS and ROBERTS, 2003).

The MMs are components of an internal, pigmented cell system in the liver and spleen tissues of some fishes, anurans and reptiles (SCALIA, GEREMIA, CORSARO et al., 1988; MICALÉ and PERDICHIZZI, 1990; CHRISTIANSEN, GRZYBOWSKI and KODAMA, 1996; MANERA, SERRA, ISANI et al., 2000; SICHEL, SCALIA and CORSARO, 2002; ZIERI, TABOGA and OLIVEIRA, 2007; MOURA, SANTOS, BELLETTI et al., 2009). According to Frye (1991), these cells are numerous in amphibians and reptiles, except among snakes, in which they are less plentiful (HACK and HELMY, 1964). These cells have various functions among them synthesis of melanin, fagocytosis and neutralization of free radicals (GUIDA, GALLONE, MAIDA et al., 2000; SICHEL, SCALIA and CORSARO, 2002).

Vertebrate melanocytes and MMs differ in the functioning of their synthetic pathways and transductional controls for melanin synthesis, as well as in the chemical composition of their melanin-bearing somes (melanosomes) (GALLONE, GUIDA, MAIDA et al., 2002; GUIDA, ZANNA, GALLONE et al., 2004). Sichel, Scalia, Mondio et al. (1997) proposed a new classification for pigmented cells in vertebrates. In this classification, besides the cells derived from the mesenchyme of the neural crest and neural tube, a third cell line that synthesizes melanin is considered to



**Figure 3.** Photomicrographs of the liver of and biliary vesicle of *T. torquatus*. a) Hepatocytes with vacuolated cytoplasm (arrow) and melano-macrophage centers (large arrow). (HE). b) Hepatocytes weakly reactive to PAS (arrow) and melano-macrophage centers (\*). PAS. c) Hepatic capsule (arrow), reticular stroma (\*) and melano-macrophage centers (large arrow). (GR). d) Biliary vesicle with simple cylindrical epithelium (arrow), lamina propria (\*) with collagen fibers and smooth muscle fibers, bile ducts (large arrows) and branch of portal vein (V). (HE). Bar: 50  $\mu$ m.

derive from the hematopoietic stem cells. These include the Kupffer cells of amphibians and reptiles (AGIUS, 1980; ZUASTI, JARÁ, FERRER et al., 1989; ZUASTI, FERRER and AROCA, 1990).

Lamers and De Haas (1985) further emphasized the close relationship of MMCs with the lymphoid cells and suggested they were the major sites of long-term antigen retention. It has been suggested on both functional and structural grounds that melano-macrophage centers may represent the primitive analogues of the germinal centers of the lymph nodes of birds and mammals (FERGUSON, 1976). Clinical studies have shown the association of MMCs with a range of highly resistant intracellular bacteria and parasites (ROBERTS, 2001). This information can help clarify the reason we observed a large quantity of MMCs in the liver of *T. torquatus*, because we found parasites present in the lumen of the gastrointestinal tube of all the specimens examined.

### 3.3 Biliary vesicle

The gallbladder is located on the visceral surface of the right lobe (Figure 1). It lies in a hollow and only its apex extends over the surface of the liver. These findings are similar to those described for other reptiles

(BANKS, 1992; BACHA and BACHA, 2003; GARDNER and OBERDÖRSTER, 2006; MARYCZ, KLEĆKOWSKA-NAWROT, MAKSYMOWICZ et al., 2009) and mammals (SAMUELSON, 2007).

The mucosa of the biliary vesicle of *T. torquatus*, like in mammals, is covered by a simple cylindrical epithelium covering the lamina propria, composed of weak connective tissue and smooth muscle fibers (Figure 3d). Only the lamina propria had a (weak) positive reaction to PAS. The same result was observed in *P. expansa* and *P. geoffroanus* by Moura, Santos, Belleti et al. (2009) but differs from that described in other reptiles, where the existence of a pseudostratified epithelium has been reported (SCHAFFNER, 1998; GARDNER and OBERDÖRSTER, 2006). The biliary system is similar to that of mammals, because the biliary canaliculi join randomly to form bile ducts. These ducts carry the bile to the biliary vesicle, where it is stored (JUNQUEIRA and CARNEIRO, 2008).

## 4 Conclusion

From the morphological description of the liver of *T. torquatus* it can be concluded that this species an excellent model for histological studies of this class.

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