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Descriptive and Comparative Histology of the Urinary System of the Hematophagous Bats *Desmodus rotundus* and *Diphylla ecaudata*

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Abstract

Desmodus rotundus and *Diphylla ecaudata*, both of which are mammals of the order Chiroptera, Desmodontidae family, their diet consisting exclusively of blood. *D. rotundus* is the main vector and transmitter of the rabies virus, which affects human beings as well as several livestock species so the study of this bat species is of high importance within the fields of animal agriculture and public health. The present study describes and compares the histologic characteristics of the urinary system of two hematophagous bat species. A total of 5 bats from each species were captured in the municipalities of Progreso de Obregón, Hidalgo (*D. rotundus*), and Huayacocotla, Veracruz (*D. ecaudata*). Organs belonging to the urinary system were extracted: kidneys, ureters, urinary bladder, and urethra; samples were fixed using 10% formalin and processed by the paraffin embedding technique, obtaining sections of 5 µm thickness, which in turn were stained using hematoxylin-eosin (H-E) and Gomori trichrome (GT) stains. From the obtained histologic preparations, a descriptive and comparative analysis of the structural organography of the urinary system of both species was made, and no noteworthy histological differences between samples were noted. The present research is intended to provide a framework for future studies of these species' currently understudied microscopic anatomy.

Keywords

Microscopic Anatomy, Vampire Bats, Kidneys, Comparative Morphology

1. Introduction

Bats are nocturnal animals, they live forming colonies in places such as caves, abandoned mines, and trees. They are the only flying mammals on the planet thanks to changes in their anatomical structure and the corresponding physiological adaptations [1]-[3]. Bats are mammals of the order Chiroptera, comprising two suborders, Microchiroptera (true bats) and Megachiroptera (flying foxes). The currently used bat classification system is based on the system developed by Miller in 1907. This system consists of 18 families, 186 genera, and more than 1400 species, of which 138 can be found in Mexico. Nonetheless, this classification constantly changes as new species are described [1]-[6]. Bats possess behavioral, anatomical, physiological, and feeding adaptations that are as interesting as they are important; bats are essential creatures in nature, not only because of their role in ecosystems they inhabit, but also because they have and continue to contribute economic and health benefits for human beings; their extraordinary adaptations have been the foundations for the development of ultrasonic communication models, insect pest control, plant pollination, seed dispensers, vaccine and pharmacological developments, and in the research of disease resistance mechanisms [2] [4] [7].

Still, little is known about the biological role of hematophagous bats, which has contributed to mankind's generalized fear of bats, which in turn has contributed to the destruction of their populations [2] [8]. Hematophagous bats feed on blood and are the only mammal species exhibiting such behavior, which is why they are also referred to as vampire bats. Because of this type of liquid diet, they have lost most of their molar teeth, and in exchange, they possess flattened, highly sharpened incisors. To feed, they select highly vascularized areas, such as the neck, throat, ear margin, hoof crowns, base of tails, nose, nipples (cattle), and inner thigh in poultry, as well as the toes and nose of human beings [2] [3] [9].

There are three species of hematophagous bats: *Desmodus rotundus*, *Diaemus youngi*, and *Diphylla ecaudata*; the first two feed mostly on blood from domestic mammals and, occasionally, humans and birds [2] [10]; the third one, *D. ecaudata*, feeds exclusively on blood from birds [2]. The three species have been linked to the transmission of the rabies virus, although currently available evidence shows that *D. rotundus* is the most relevant vector of the disease [11] [12].

The present study offers an ample histological description and comparison of the urinary system, given the importance of this system in the preservation of protein metabolism homeostasis in hematophagous bats. Considering the limited reported information regarding the histological characteristics of the uri-

nary system of hematophagous bats, this study aims for the morphological knowledge presented to contribute to the generation of new strategies for population control of these species (taking into consideration their role as rabies vectors) with a directed and rational approach, based on their particular morpho-physiological characteristics, in such a way that do not other species of bats are affected, and an ecological disaster is not caused. Also, it is important to increase our knowledge about these animals, thus preventing the perpetuation of misconceptions that could potentially result in their extinction [2].

2. Materials and Methods

2.1. Collection of Biological Samples

The procedures described in this section were approved by the UAEH's Internal Ethics Committee for the use and care of laboratory animals. Samples were obtained from 5 bats from each species (2 females and 3 males), which were captured in the municipalities of Progreso de Obregón, Hidalgo (*Desmodus rotundus*), and Huayacocotla, Veracruz (*Diphylla ecaudata*) using a mist net system [9]. Captured specimens were transported alive to the laboratory, in the shortest possible time (maximum 8 hours) to minimize the stress generated by handling (crucial to ensure the well-being of the bats), where they were euthanized administering an intrahepatic injection of 1.5 mL (94.5 mg) of sodium pentobarbital (PisabentalMR, PISA, 65 mg/mL), following what is established in the Official Mexican Norm NOM-033-SAG/ZOO-2014: Techniques for killing domestic and wild animals. Immediately after death, a necropsy of each subject was performed: a ventral midline incision was made, thus exposing both the thoracic and abdominal cavities, and the organs and structures belonging to the urinary system were identified (kidneys, ureters, bladder, and urethra); once sample sites were identified, the biological material was collected within a period of no more than 10 minutes for each bat, to maintain tissue integrity, and each sample was placed in a container filled with a 3.5% aqueous formaldehyde fixative solution, buffered with monobasic sodium phosphate and dibasic sodium phosphate [13].

2.2. Histological Processing

Biological samples were processed according to the paraffin embedding technique [13]; samples were placed in histocassettes and then placed in an automatic tissue processor (Microm, TPI020 model), inside which samples were water washed, and dehydrated with isopropanol, xylene cleared and infiltrated with histological paraffin. Afterward, samples were embedded in blocks of histological paraffin, from which 6 μm thick sections were obtained using a microtome (Leica, RM2125RT model). The resulting histological sections were collected using microscope slides and then placed in a tissue flotation water bath (Premiere, XH-1001 model). Finally, samples were placed on a hot plate (Thermolyne, 2200 model) at 60˚ Celsius for a few seconds, to attach the section to the slide [13].

2.3. Histological Staining

Tissue slides were stained either using the Hematoxylin-Eosin (H-E) or Gomori trichrome (GT) staining techniques, the latter allowing the distinction between smooth muscle collagen fibers and other histological elements [13]. Finally, slides were covered with a coverslip, applying a drop of Ultra KittMR resin (JT Baker) over the tissue.

2.4. Microscopy

Once the permanent histological preparations were obtained, they were observed using a bright field microscope (Olympus, BX41 model), with a 10× ocular lens and 4×, 10× and 40×, objective lens, for their interpretation and histological analysis.

2.5. Image Acquisition and Analysis

To capture the images a digital camera was used (Media Cybernetics, Evolution VF model), as well as the ImagePro Express 6.0MR software (Media Cybernetics), which was installed in a computer (Vaio brand, VCG-RB43MGX model, 1 Gb RAM), for image digitalization, storage and analysis in a TIFF format. Original amplifications were 40×, 100× and 400× and a size bar, properly calibrated, was added in the micrographs).

3. Results

A comparative histological evaluation of the urinary systems of the blood-feeding bats *Desmodus rotundus* and *Diphylla ecaudata* was conducted. The kidneys are a pair of tissue organs consisting of a stroma and a parenchyma. The kidney's stroma is made up of a very thin outer capsule composed of dense irregular collagenous connective tissue. It does not form trabeculae (**Figure 1(A)**). The kidney's parenchyma consists of the cortex and medulla (**Figure 1(A)** and **Figure 1(B)**). The renal pelvis is located within the medulla. The renal cortex contains renal corpuscles that are distributed randomly (**Figure 1(C)** and **Figure 1(D)**), each surrounded by a glomerular capsule, which has two layers: the parietal (outer) layer and the visceral (inner) layer (**Figure 1(F)**). The inner layer is closely related to the inner capillary vessels, forming the renal glomerulus within each renal corpuscle (**Figure 1(F)**).

Each glomerulus originates from an afferent arteriole and drains into an efferent arteriole. Both blood vessels are located near the vascular pole of each corpuscle, where the juxtaglomerular apparatus is found. This apparatus consists of juxtaglomerular cells and the macula densa (**Figure 1(E)**). At the opposite pole, known as the urinary pole, the glomerulus is connected to the proximal convoluted tubule (**Figure 1(F)**), which is lined with a simple cuboidal epithelium with microvilli (also called a brush border). The cells of the proximal convoluted tubule are highly acidophilic (**Figure 1(C)**). Similarly, the distal convoluted tubules also have a simple cuboidal epithelium with microvilli, although

these are not easily visible under optical microscopy, and their cells are less acidophilic (Figure 1(C)).

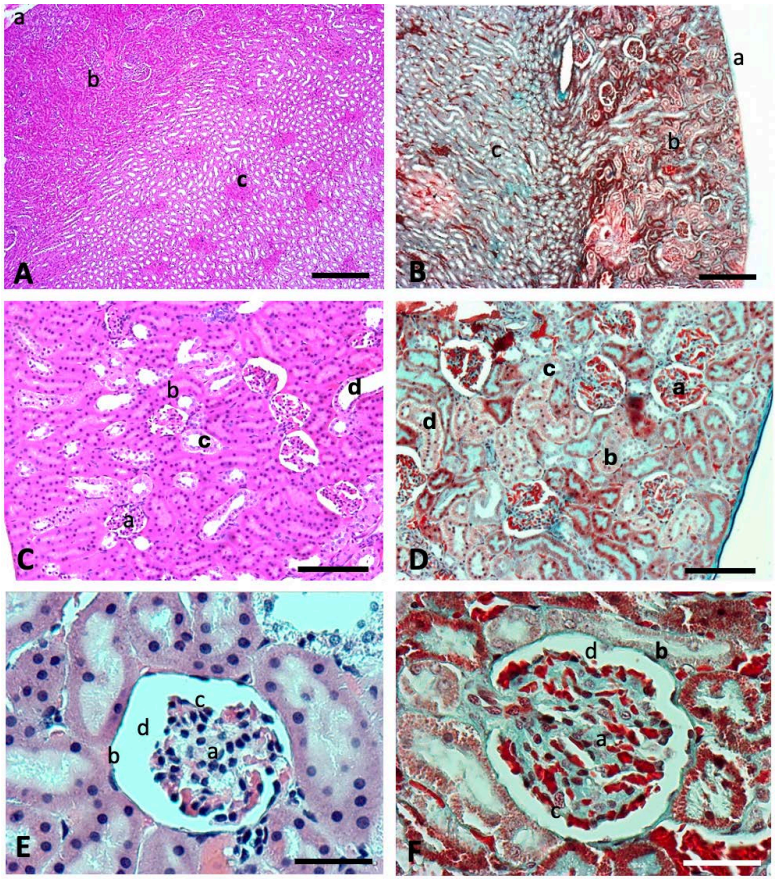

Figure 1. Kidney. 40×, A. *Desmodus rotundus*. B. *Diphylla ecaudata*. The kidney consists of a stroma and a parenchyma. The stroma is composed of a very thin outer capsule (a). The kidney's parenchyma consists of an outer cortex (b) and an inner medulla (c). Bars: 100 µm. 100×. C. *Desmodus rotundus*. D. *Diphylla ecaudata*. The renal cortex presents randomly distributed renal corpuscles (a), proximal convoluted tubules (b), distal convoluted tubules (c) and collecting ducts (d). Bars: 50 µm. 400×. E. *Diphylla ecaudata*. F. *Desmodus rotundus*. Renal corpuscles consist of a glomerular capsule and renal glomerulus (a). The glomerular capsule consists of a parietal layer (b) and a visceral layer (c). Between these layers a capsular space is observed (d). Bars 15 µm. A, C and E. H-E stain. B, D and F. T. Gomori stain.

The collecting ducts have a simple cuboidal epithelium and microvilli that are not easily visualized (Figure 2(D)). The renal cortex is irrigated by numerous blood vessels, including interlobular arteries and veins arising from the arcuate arteries and veins, located at the border of the renal cortex and the renal medulla (Figure 2(A) and Figure 2(B)). The renal medulla contains straight tubules (both descending and distal ascending) with corresponding thick and thin portions, forming the nephron's loop (Figure 2(E) and Figure 2(F)). Both structures exhibit a simple cuboidal epithelium with microvilli in their thick segments and a simple squamous epithelium in their thin portions. Thin-walled blood

vessels corresponding to the interlobar vessels are observed in the renal medulla, branching out from the renal vein and artery. The renal pelvis within the renal medulla presents a transitional epithelium (Figure 2(C) and Figure 2(D)). In some sections, the renal vein and artery are also observed.

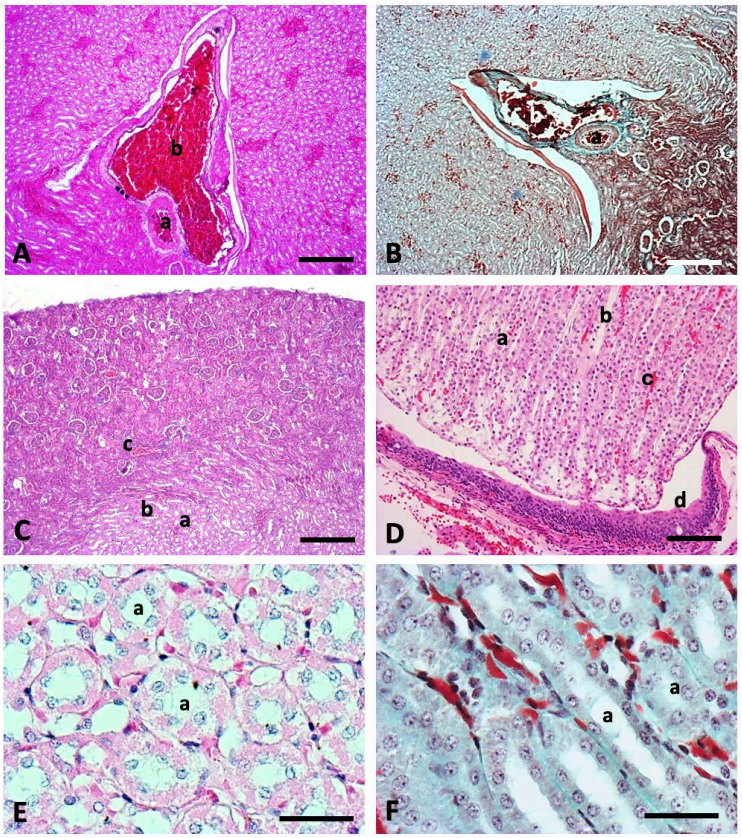

Figure 2. Border zone between the renal cortex and the renal medulla. 40×. A. *Desmodus rotundus*. B. *Diphylla ecaudata*. Arciform artery (a) and venous (b). Bars: 100 µm. 40×. C. *Desmodus rotundus*. D. *Diphylla ecaudata*. The renal medulla presents the straight descending and ascending tubules (a) and the collecting ducts (b). Interlobar vessels are also observed (c), these branch out from the renal vein and artery. In D is observed the renal pelvis (d), whose epithelium is a transitional epithelium as it integrates to the ureter. Bars: 100 µm. 400×. Renal medulla. E. *Desmodus rotundus*. F. *Diphylla ecaudata*. Displays straight ascending and descending tubules (a). A, C, D and E. H-E stain. B and F. Gomori's T. stain. Bars: 15 µm.

The ureters are paired organs with a tubular structure that carry urine from the kidneys to the bladder. They consist of three main layers: mucosa, muscularis, and adventitia (Figure 3(A) and Figure 3(B)). The mucosa is made up of transitional epithelium and a lamina propria of loose collagenous connective tissue (Figure 3(D)). The muscular layer is divided into three layers: internal longitudinal, middle circular, and external longitudinal, all composed of smooth muscle and difficult to differentiate from one another (Figure 3(C)). The adventitia is composed of loose collagenous connective tissue and brown adipose tissue and contains numerous arteries, venules, and nerves (Figure 3(D)).

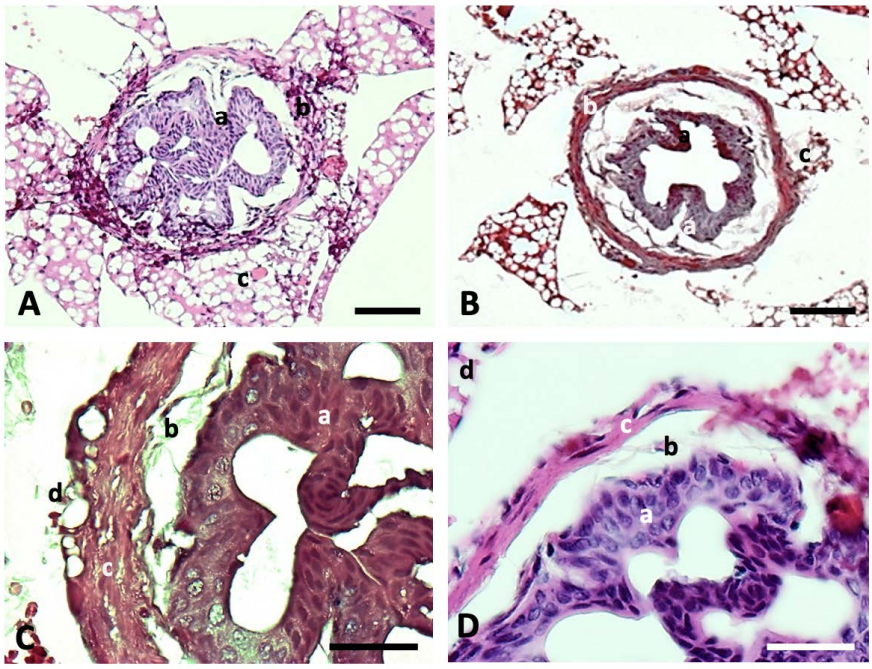

Figure 3. Ureter. 100×. A. *Desmodus rotundus*. B. *Diphylla ecaudata*. Displays three layers: mucosa (a), muscularis (b) and adventitia (c). Bars: 50 µm. 400×. C. *Desmodus rotundus*. D. *Diphylla ecaudata*. The mucosa layer is formed by a transitional epithelium (a) and a lamina propria (b). The muscular layer (c) is thin. The adventitia consists of loose collagenous connective tissue and brown adipose tissue (d). Bars: 15 µm. A and D. H-E stain. B and C. Gomori's T. stain.

The urinary bladder is a hollow muscular organ with three main layers: the mucosa, the muscularis, and the serosa (Figure 4(A) and Figure 4(B)). The mucosa consists of a transitional epithelium and a layer of loose collagenous connective tissue known as the lamina propria, which is arranged in folds called rugae (Figure 4(C) and Figure 4(D)). The muscularis is very thin and has three layers of smooth muscle: the internal longitudinal layer, the middle circular layer, and the external longitudinal layer (Figure 4(A)). The serosa is made up of loose collagenous connective tissue and a mesothelium of simple squamous epithelium (Figure 4(A)).

The urethra is a tube-like organ that connects the bladder to the outside of the body. In females, it typically opens into the vestibule in the pelvic floor, and in males, it ends at the tip of the penis, called the urethral meatus. The urethra has four layers: mucosa, submucosa, muscularis, and adventitia (Figure 5(A), Figure 5(B)). The mucosa is made up of transitional epithelium and a highly vascularized lamina propria of loose collagenous connective tissue with associated elastic fibers (Figure 5(D)). The submucosa is also highly vascularized and consists of loose collagenous connective tissue (Figure 5(C), Figure 5(D)). The muscular layer has an inner circular layer and a thinner outer longitudinal layer, both made of smooth muscle. The adventitia is composed of loose collagenous connective tissue (Figure 5(A), Figure 5(B)).

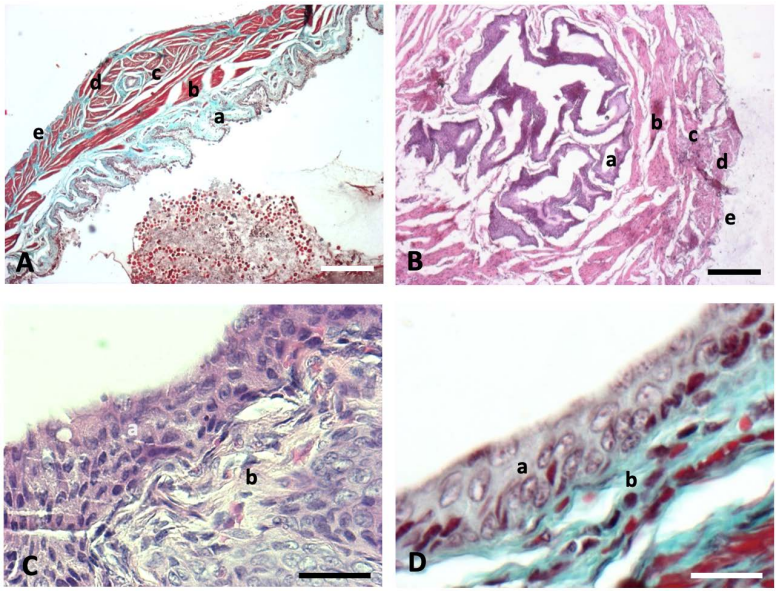

Figure 4. Bladder. 40×. A. *Desmodus rotundus*. B. *Diphylla ecaudata.* The bladder's wall consists, from the inside out, of a tunica mucosa (a). Underneath the mucosa is the tunica muscularis, which presents three layers of its own: internal longitudinal (b), middle circular (c) and external longitudinal (d). Externally, presents the tunica serosa (e), which consists loose collagenous connective tissue and a mesothelium. Bars: 100 μm. 400×. C. *Desmodus rotundus.* D. *Diphylla ecaudata.* The bladder wall consists, from the inside out, of three layers: a fold-forming tunica mucosa that is composed of a transitional epithelium (a) and a lamina propria (b). Bars: 15 μm. A and D. Gomori's T. stain. B and C. H-E stain.

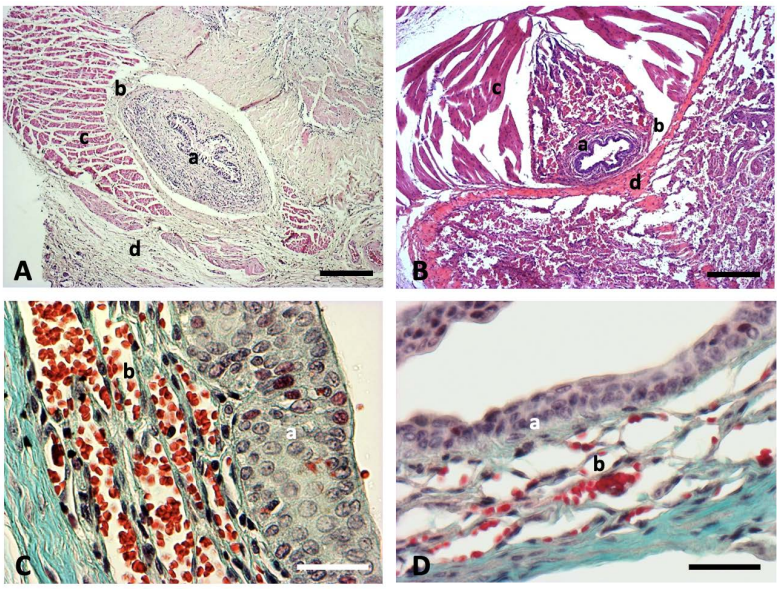

Figure 5. Urethra. 40×. H-E. A. *Desmodus rotundus*. B. *Diphylla ecaudata.* It presents four layer: tunica mucosa (a), tela submucosa (b), tunica muscularis (c) and tunica adventitia (d). Bars: 100 μm. 400×. C. *Desmodus rotundus*. D. *Diphylla ecaudata*. The tunica mucosa consists of a transitional epithelium (a) and a lamina propria (b) of collagenous connective tissue and associated elastic fibers, a highly vascularized capillary plexus is found within the lamina propria. Bars: 15 μm.

4. Discussion

Bats (Mammalia, order Chiroptera) make up the second largest group of mammals, after Rodentia order [6] [14]. Their ability to fly, adapt, and inhabit various environments has contributed to their evolutionary success [15]. The urinary system of hematophagous bats, due to their unique feeding habits, is a crucial aspect of their survival. Despite this, the histological features of this system show no significant differences between the hematophagous bats *Desmodus rotundus* and *Diphylla ecaudata* in their general structure. This high functionality is of particular interest, as the changes in renal function required to accommodate their blood diet are not yet understood [1] [16]-[18].

In this context, we highlight the specific morphological characteristics discovered and their potential significance. It's important to note that no notable histological differences were observed between the two species. In a previous comparative study, no morphometric differences in the glomerular area were observed between *D. rotundus* and *D. ecaudata* [18].

The kidneys of these bats have a thinner capsule compared to other mammals, likely due to their small size. However, this is not the case for similarly sized mammals like mice. It's important to note that the kidney size of these bats in relation to their body size is proportionally larger than in other mammals [17]. It has been shown that the kidneys of hematophagous bats represent a greater mass in relation to body size, associated with a greater workload that the organ receives, which influences a greater volumetric density of the glomeruli and glomerular area, which in turn generates greater filtration of protein metabolites, especially urea. Likewise, it has been identified that these animals have a greater production of nitric oxide and greater glutathione S-transferase activity, and therefore a better capacity for reducing iron in the plasma and cellular defense against increased lipid peroxidation, to preserve kidney function, which reinforces the role of these adaptive alterations in the survival of hematophagous bats [19].

Furthermore, there are reports of increased glomerular area and volumetric densities of the glomerular and urinary tubules in adult *D. rotundus* compared to younger bats. This has been linked to changes in the diet of adult animals compared to young ones. Neonate bats feed on milk and this diet provides them with a balanced portion of carbohydrates, lipids, and proteins, as well as vitamins and minerals necessary for their growth and development. However, the protein content of milk varies with the mother's protein intake and is much lower than the protein content of blood, making the transition of feeding during weaning critical for the survival of the offspring. Therefore, there are gradual morphological changes in the kidneys that allow them to adapt to their unique blood diet as adults. These changes include increased values of the renal somatic index, renal volume, medulla/internal cortex, and medulla/total cortex ratios, as well as a plasticity of the pedicels in the glomerular membrane, caused by the adaptive changes associated with the change in diet: hypertension glomerular,

hyperfiltration and hypertrophy of renal tissue [18]. Besides, analysis of the stomach contents of neonates indicates that, from the first hours of life until weaning (three to seven months of age), mothers regurgitate blood to feed them. This results in a mixture of milk and blood in the stomach. The purpose is to facilitate a gradual adaptation of the stomach to the blood, and to supply the microbiota necessary for the blood digestion process [20]. After weaning, they specialize in a strict blood diet [2].

The renal pelvis has a simple squamous or simple cuboidal epithelium, instead of the more typical transitional epithelium. However, a transitional epithelium is present at the level of the ureter, bladder, and urethra. This difference may be due to the animals' ability to reabsorb water from already-formed urine. Water reabsorption into the renal tubules may be facilitated by this thinner epithelium [21] [22]. This mechanism is an osmotic process dependent on urea concentration. The thickness of the epithelium is expected to play a significant role in the proper function of this mechanism, given that urea concentration in these animals is higher due to their protein-rich food source [1] [21].

In 2008, [23] reported finding loopless nephrons (reptilian type, according to their classification) in insectivorous bats, although the specific species was not mentioned. The present study confirms the presence of structures corresponding to the ascending and descending, thick and thin portions of the nephron's loop. Additionally, no scientific record reporting the absence of nephron loops in the histology of vertebrates was found [24].

The renal morphophysiology conditions observed in vampire bats may be related to a high demand for excretion of nitrogenous products due to protein and iron overload, in comparison to fruit-eating species [19]. In terms of the urinary tract, no histological differences from those described in other mammals were found, except for the presence of a highly vascularized capillary plexus in the urethra, which is not found in other animals [16] [25]. This plexus was observed in samples from both *D. rotundus* and *D. ecaudata*.

It remains to be seen whether such a plexus is present in other bat species, but it could serve as an additional mechanism for water reabsorption in these species. This may enhance the capacity for urine concentration under certain circumstances, supporting the organism in maintaining its hydroelectrolytic homeostasis.

It would be valuable to acquire data for the third known species of blood-sucking bat, *Diaemus youngi*. This data will help us conduct a comparative morphological study to better understand the kidney function of hematophagous bats. These bats are the only small mammals that can metabolize the high levels of proteins they ingest without any signs of kidney damage.

It is recommended to conduct a comparative study of the histological characteristics of non-hematophagous bats. This study aims to determine whether the observed differences in kidney structure are partially influenced by the animals' dietary habits.

5. Conclusion

The present study is the first to provide a detailed histological and comparative description of the urinary system of two species of hematophagous bats: *Desmodus rotundus* and *Diphylla ecaudata*. The results indicate that there were no histological differences between the two species, and the overall tissue structure of the urinary organs was like that of other mammals, except for the epithelium of the renal pelvis and the lamina propria of the urethra, which showed some differences. This morphological data presented here will help us better understand the evolutionary adaptations that enable these species to survive exclusively on a blood-based diet from other animals, so that this information in turn is a solid scientific basis for the design of population control strategies, among others, aimed at preventing the transmission of diseases from these animals to humans, but with a friendly approach for other species of bats and the environment in general, within the One Health concept.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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