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Comparative Study on the Kidneys of *Ptychadena mascarenensis* and *Chalcides ocellatus ocellatus* in Relation to Their Environment

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ABSTRACT

Background: The kidneys manage body fluids and electrolytes in addition to performing osmoregulation and metabolic waste disposal. The goal of the present study was to investigate the structural characteristics of the kidneys of two tetrapod species; *Ptychadena mascarenensis* (Mascarene frog) and *Chalcides ocellatus ocellatus* (Ocellated skink) concerning their habitat, which could be linked to unique structural renal differences.

Methods: During December 2020, four specimens of each species were obtained from various locations in Egypt. The animals were captured alive and were suffocated by inhaling chloroform. There kidneys were removed and processed for morphological, histological and electron microscopy examinations.

Result: *Ptychadena mascarenensis* and *Chalcides ocellatus ocellatus* have groups of nephrons in their kidneys and each nephron consists of a renal corpuscle, an intermediate segment, a proximal tubule, a distal tubule and a collecting duct system. The renal corpuscles and tubules were dispersed throughout the cortex and medulla of both species so the cortex and medulla were not differentiated. In the two tetrapod classes Amphibia (*Ptychadena mascareniensis*) and Reptilia (*Chalcides ocellatus ocellatus*), some differences in the morphological, histological and ultrastructural features of the kidney have been identified. Animals use each of these structures to help them adapt to their surroundings.

Key words: Environment, Kidney, Nephron.

INTRODUCTION

The form and function of distinct vertebrate kidneys vary, though; these variations allow the organs to be adapted to the environment in which the animals dwell (Kimball, 2023). Osmoregulation and metabolic waste removal were all performed by the kidneys in addition to maintaining body fluids and electrolytes (Ross *et al.,* 2003).

The nephron was the fundamental functional unit of all three kidney forms; pronephros, mesonephros and metanephros, despite differences in overall organization and complexity (Saxe'n, 1987). The two primary functional parts of the kidney were the blood-vascular system and nephrons. The renal glomeruli were made up of overlapping capillaries that were connected and create many lobules with, typically, one afferent and one efferent arterioles **(**Kikuta and Murakami, 1989). Glomeruli were supplied by a single afferent arteriole and discharged into peritubular arteries via a single, thinner efferent arteriole (Lametschwandtner, 2020).

The current study is to investigate the kidney structures and functions concerning their habitats in an amphibian (*Ptychadena mascareniensis*) and a reptile (*Chalcides ocellatus ocellatus*).

MATERIALS AND METHODS

Ptychadena mascareniensis has been found in Egypt's Nile Valley and Delta. It lives in densely vegetated irrigation, drainage canals, swamps, marshes and pans (Harper *et al.,* 2010). It was collected from Egypt's Beheira Governorate. This frog gets its name from two light lines running along its back. On its dorsum, it has some black squarish marks. It was around 4.3 cm in length and 9.44 gm in weight. It eats terrestrial beetles, bugs, spiders, soil worms and snails and is a carnivore (Measey *et al.,* 2009).

The Mediterranean Coastal Desert and Sinai, as well as the Nile Valley and Delta, were all home to *Chalcides ocellatus ocellatus* in Egypt. These can be found in sandy desert to the banks of Nile Valley and Delta irrigation canals (Saleh, 1997). These were collected from Giza, Egypt, in

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the village of Abou Rawash. Its length was approximately 12.4 cm and its weight was 17.77 gm. The ocellated skink gets its name from the numerous black and white ocelli, or "eye markings," that create bands over the back of the animal. Coleoptera, Dermaptera, Hymenoptera, Odonata and Orthoptera are among the insects it preys on. It also has insect larvae, lizards and plants in its stomach (Dylan *et al.,* 2012).

Amphibians were aquatic animals that live in freshwater. They osmotically absorb water and lose dissolved substances. To maintain a body fluid osmolarity that was significantly greater than that of environmental fresh water, they excrete diluted urine (Stoner, 1977). Since many reptiles inhabit dry habitats, their glomeruli were often relatively tiny and some reptiles even lack any glomeruli at all. Those with glomeruli only filter the amount of water necessary to flush the uric acid that the tubules release into the cloaca. In the cloaca, the majority of this moisture was reabsorbed (Kimball, 2023). Two of the most significant issues facing animals existing in the dry zone were water supply and conservation. The animals in this environment must manage their water balances by employing some behavioral and physiological techniques (Davis and DeNardo, 2010; Wilms *et al.,* 2009; Beck and Jennings, 2003).

During December 2020, four specimens of each species were obtained from various locations in Egypt. The animals were captured alive and transported to the histology laboratory, Department of Zoology and Entomology, Faculty of Science, Helwan University. They were suffocated by inhaling dimethyl ether, the animals were dissected and there kidneys were removed. The length and weight of all kidneys were measured and photographed for morphological description. Specimens were processed for histological examination and stained with Hematoxylin and eosin according to Drury and Wallington (1980). Using a Leica optic photomicroscope, the sections were examined and photographed. At the Transmission Electron Microscope Unit, Faculty of Science, Ain-Shams University, the sections were analyzed and photographed with a JEOL JEM-1200 EX II Electron Microscope.

RESULTS AND DISCUSSION

The kidneys of *Ptychadena mascareniensis*, were paired and situated posterodorsally in the abdominal cavity (Fig 1A). They were medium in size, reddish-brown in color and cylindrical in shape. Their length and weight were respectively 1.3 cm and 0.015 gm. They were non-capsulated and have segmental constrictions in the frontal region that split the organ into smaller divisions (Fig 1C). Similar results were observed in *Geotrypetes seraphini* kidneys (Møbjerg *et al.,* 2004). In contrast to these findings, adult *Xenopus laevis* (a pipid frog) possessed kidneys that were enclosed in a thin connective tissue capsule (Lametschwandtner, 2020).

The ocellated skink has kidneys that were located in the back of the trunk (Fig 1B). The kidneys were flattened, symmetrical, elongated, paired and dorsoventrally located. They were encased in capsules, triangular in shape, small, reddish-brown in color and silky in texture. They were 0.8 cm long and weight 0.065 gm (Fig 1C). The same outcomes were observed in the kidneys of *Uromastyx acanthinura* (Talmatamar *et al.,* 2020), *Notomabuya frenata* (Novelli *et al.,* 2018) and *Cerberus rynchops* (Thongboon *et al.,* 2017). The cortex and medulla were not differentiated in both species, this means that the renal corpuscles and tubules were scattered throughout the cortex and medulla. Al-Shehri and Al-Doaiss (2021), Talmatamar *et al.* (2020), Novelli *et al.* (2018), Thongboon *et al.* (2017) and Yari and Gharzi (2013) all achieved similar findings.

Histologically, the Mascarene frog's kidney was made up of groups of nephrons; each nephron is made up of a renal (Malpighian) corpuscle, a neck segment, an intermediate segment, renal tubules (proximal and distal tubules) and collecting duct system. In the kidneys of *Geotrypetes seraphini* and *Triturus pyrrhogaster*, Møbjerg *et al.* (2004) and Sakai and Kawahara (1983) showed similar results. The components of the nephrons in the renal parenchyma of *Chalcides ocellatus ocellatus* were clearly defined (the renal corpuscles, the proximal convoluted tubule, the intermediate segment, the distal convoluted tubules, the connecting tubule and the collecting duct system). According to Al-Shehri and Al-Doaiss (2021), similar results were obtained.

In both species, the renal corpuscle was made of two structures; the bowman's capsule, a layer of squamous epithelium, encircles the vascular loops of the glomerulus and the bowman's space refers to the area between the glomerulus and the bowman's capsule (Fig 2A and 3C). At the TEM level, the visceral layer (the inner layer) of the bowman's capsule, which is made up of podocytes and surrounds the glomerular capillaries and the thin parietal layer (the outer layer), which was continuous with the epithelium of the renal tubule, were the two components of the bowman's capsule. Al-Shehri and Al-Doaiss (2021) reported the same observations for reptiles and Sakai and Kawahara (1983) reported the same findings for amphibians. The podocyte epithelium, which was found in the visceral layer of the capsule, was made up of interdigitating cell processes that produce foot processes and cell bodies that contain the nucleus. These alternating foot processes create small, filtered openings that were connected by diaphragms and sit on the thick basement membrane. The endothelium of the glomerular capillaries has fenestrae of various sizes. The capillary lumen was bulged by the endothelial cell bodies, which contain nuclei. On the basement membrane's endothelial side, there were mesangial cells. Thus, the glomerular basement membrane, the foot processes of the podocytes and the fenestrated endothelium of the glomerular capillaries make up the filtration barrier (Fig 2C and D and 4A and B). Sakai and Kawahara (1983) reached the same conclusions. According to Ruppert (1994), ultrafiltration in amphibians takes place in the glomerulus. As in other vertebrates, the Malpighian corpuscle's three-layered barrier

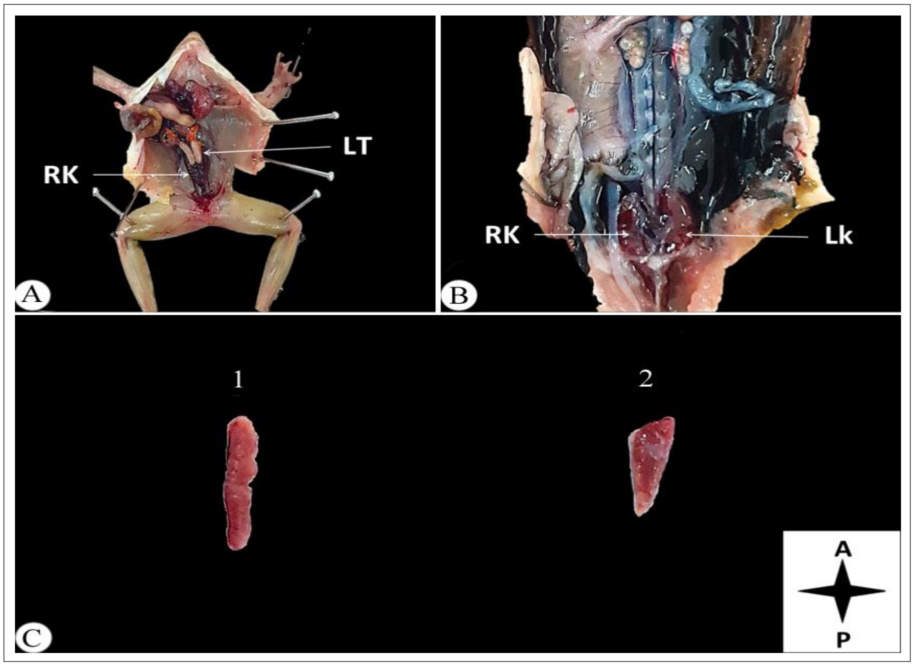
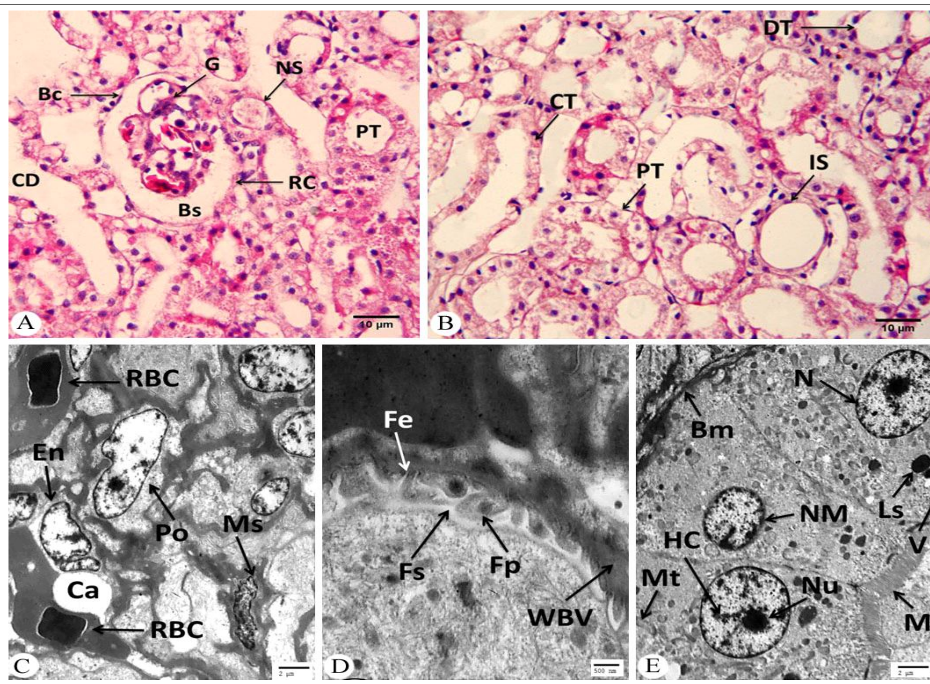
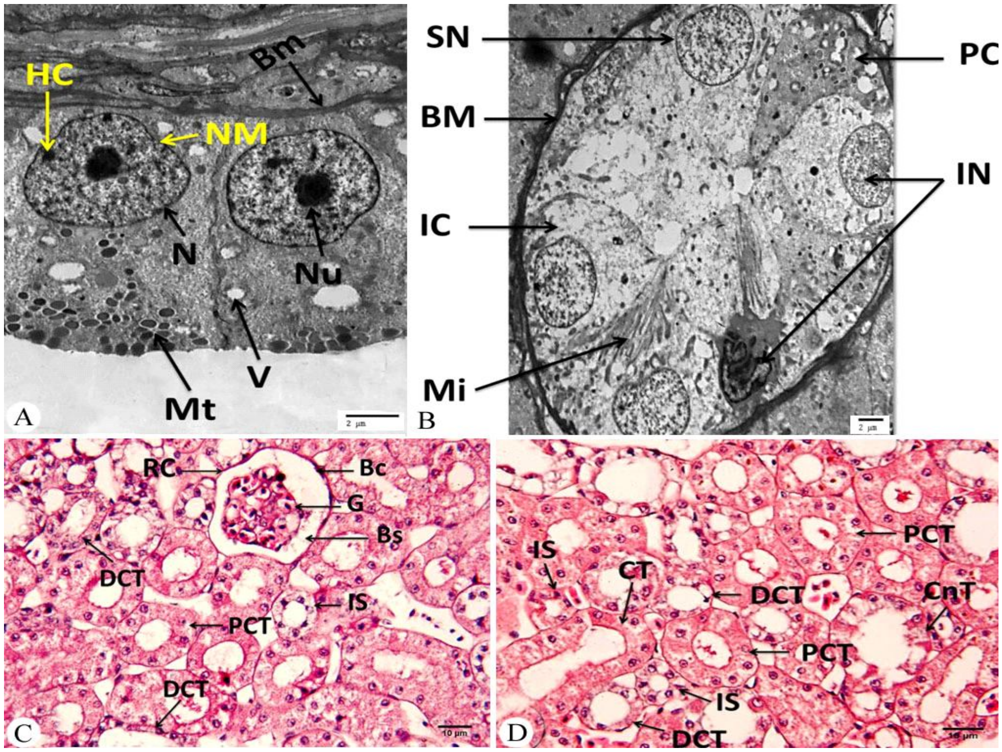


Fig 1: (A) photomacrograph showing the right kidney (RK) and the left testis (LT) of *Ptychadena mascareniensis*; (B) photomacrograph of the right kidney (RK) and the left kidney (LK) which is positioned in the posterior section of the trunk of *Chalcides ocellatus ocellatus*; (C) photomacrograph of the isolated kidneys of: (1) *Ptychadena mascareniensis* and (2) *Chalcides ocellatus ocellatus*.



(A) the renal corpuscle (RC) including the bowman's capsule (Bc), the glomerulus (G), the bowman's space (Bs); (B) the neck segment (NS), the intermediate segment (IS), the proximal tubule (PT), the distal tubule (DT), the collecting tubule (CT) and the collecting duct (CD). (C and D) the glomerulus illustrating the red blood cell (RBC), the podocyte (Po), the mesangial cell (Ms), the endothelial cell (En), the capillary (Ca), the fenestration (Fe), the foot processes (Fp), the filtration space (Fs) and the wall of blood vessel (WBV); (E) the proximal tubule illustrating the basement membrane (Bm), the nucleus (N), the nucleolus (Nu), the nuclear membrane (NM), the heterochromatin (HC), the mitochondria (Mt), the microvilli (Mi), the lysosomes (Ls) and the vacuole (V).

Fig 2: Photomicrographs of longitudinal sections of the kidney of *Ptychadena mascareniensis* stained with H&E.



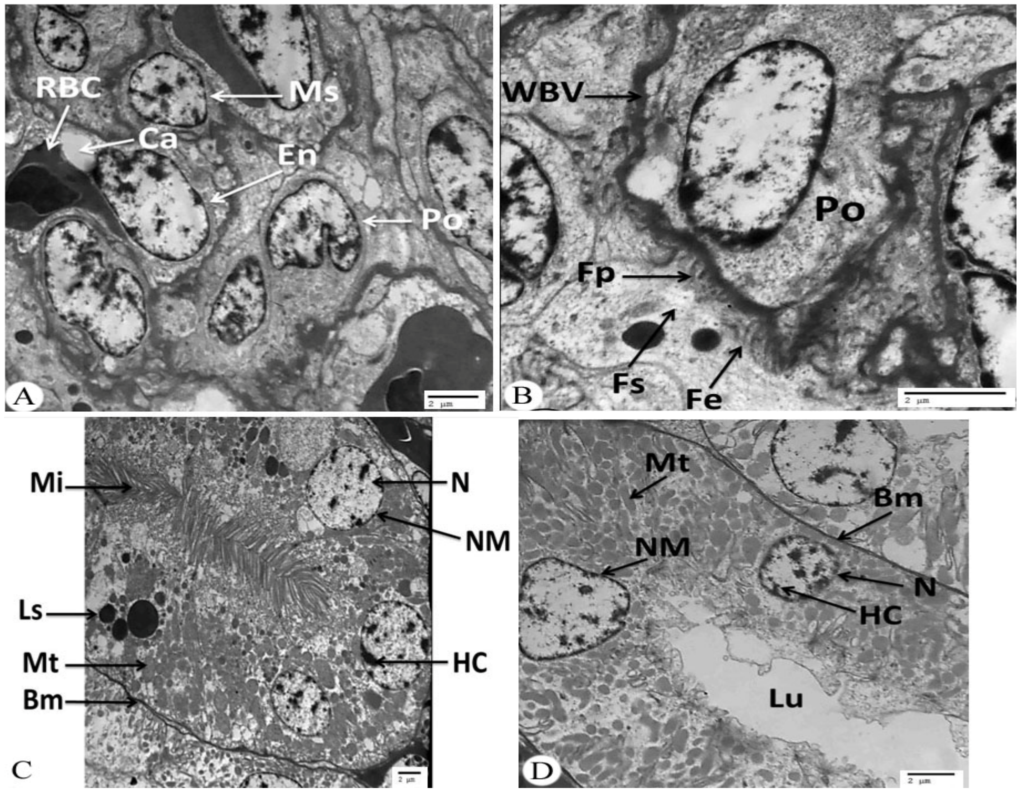
(A) the distal tubule illustrating the basement membrane (Bm), the nucleus (N), the nucleolus (Nu), the nuclear membrane (NM), the heterochromatin (HC), the mitochondria (Mt) and the vacuole (V); (B) the collecting tubule illustrating the basement membrane (Bm), the principle cell (PC), the intercalated cell (IC), the spherical nuclei (SN), the irregular nuclei (IN) and the microvilli (Mi). (C and D) A photomicrographs of longitudinal and transverse sections of the kidney of *Chalcides ocellatus ocellatus* stained with H&E showing the renal corpuscle (RC), the bowman's capsule (Bc), the glomerulus (G), the bowman's space (Bs), the proximal convoluted tubule (PCT), the intermediate segment (IS), the distal convoluted tubule (DCT), connecting tubule (CnT) and the collecting tubule (CT).

Fig 3: Transmission electron micrographs of *Ptychadena mascareniensis* kidney.

serves as a filter for primary urine to form. According to Richter and Splechtna (1996) and Melman *et al.* (1991), the amphibian Malpighian corpuscle has undergone extensive studies and was quite large when compared to that of other vertebrates. Talmatamar *et al.* (2020) showed that *Uromastyx acanthinura's* kidney's glomeruli were small in comparison to frogs. This could provide information on the *Uromastyx acanthinura's* urine flow rate and glomerular filtration rate. The minute size of glomeruli has been highlighted as a crucial method for water conservation by lowering the glomerular filtration rate and urine flow rate (Mbassa, 1988; Gambarian, 1994).

Ptychadena mascarenensis's kidney has a neck segment (Fig 2A) and an intermediate segment (Fig 2B) made up of low cuboidal or flat, ciliated cells. The intermediate segment was lined similarly to the proximal convoluted tubule in *Chalcides ocellatus ocellatus's* kidney, but it was smaller and lacks brush-bordered cells (Fig 3C and D, 5A). The neck segment and the intermediate segment in amphibian nephron were involved in fluid propulsion (Hentschel and Elger, 1989). According to Møbjerg *et al.* (2004), this may include caecilians and salamanders absorbing liquid from the body cavity through peritoneal funnels that open into the neck segment. According to Møbjerg *et al.* (1998) and Uchiyama *et al.* (1990), the nephron in anurans lost its attachment to the coelom and the peritoneal funnels open into the peritubular arteries surrounding the nephrons. The intermediate segment in the kidney of the lizard *Uromastyx acanthinura* was small and situated similarly to the loop of Henle in mammals and birds (Talmatamar *et al.,* 2020; Hentschel and Elger, 1989). Gabri (1983) asserts that this tubule exhibits a low absorptive capacity. Lower vertebrates may need cilia present in the intermediate segment to attain ideal ultrafiltrate flow rates along the distal tubule (Peek and McMillan, 1979).

The proximal tubule in each examined species was homocellular and composed of brush-boarded cuboidal epithelial cells called principal cells, which, based on the basement membrane, carry numerous microvilli on their apical surfaces and have a narrow lumen (Fig 2 A and B, 3 A and B and 5 C). According to TEM images, a huge, regular, spherical nucleus located in the center of the cell can be



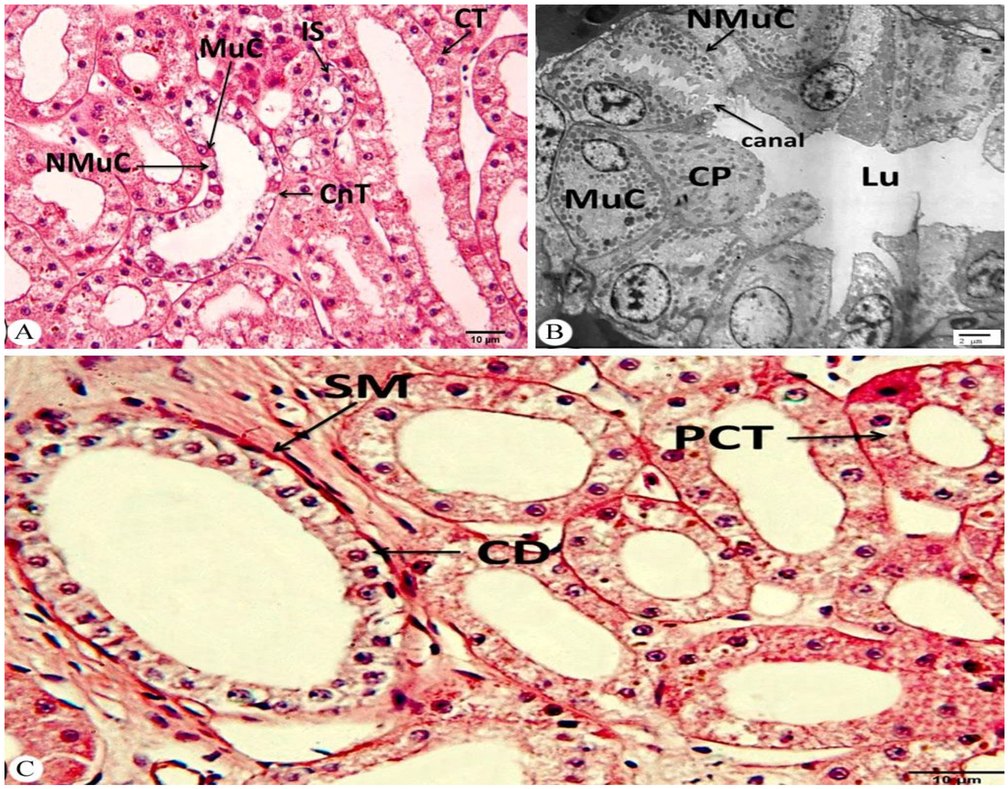
(A) the glomerulus illustrating the red blood cell (RBC), the podocyte (Po), the mesangial cell (Ms), the endothelial cell (En) and the capillary (Ca); (B) the glomerulus illustrating the podocyte (Po), the fenestration (Fe), the foot processes (Fp), the filtration space (Fs) and the wall of blood vessle (WBV); (C) the proximal convoluted tubule illustrating the basement membrane (Bm), the nucleus (N), the nuclear membrane (NM), the heterochromatin (HC), the mitochondria (Mt), the microvilli (Mi) and the lysosomes (Ls); (D) the distal convoluted tubule illustrating the basement membrane (Bm), the nucleus (N), the nuclear membrane (NM), the heterochromatin (HC), the mitochondria (Mt) and the lumen (Lu).

Fig 4: Transmission electron micrographs of *Chalcides ocellatus ocellatus* kidney.

seen. The cells of PCT contain a great quantity of heterochromatin. Each cell contains a large number of lysosomes of various sizes and numerous elongated mitochondria were found as well as some vacuoles (Fig 2E and 4C). Al-Shehri and Al-Doaiss (2021) reported the same result. According to Møbjerg *et al.* (2004), Møbjerg *et al.* (1998) and Farias *et al.* (1998), the amphibian proximal tubule cells were specifically designed for the uptake of an isotonic absorbate. This comprises the development of a luminal brush border and lysosomal system on an ultrastructural level. The proximal convoluted tubule in *Chalcides ocellatus ocellatus* was used to provide a larger surface for salt and water reabsorption. To avoid dehydration caused by the arid environment, the animal must conserve body fluids. According to Talmatamar *et al.* (2020), the same outcomes were observed in *Uromastyx acanthinura*.

The distal tubule in both species was made up of simple cuboidal epithelial cells with a large lumen that rests on a basement membrane (Fig 2B and 3C and D). The nucleus was spherical to ovoid in form, placed basally towards the basement membrane and has the appropriate quantities of heterochromatin. At the TEM level, there were a large number of elongated mitochondria distributed throughout the cell. The cells display cytoplasmic protrusions only in the kidney of *Chalcides ocellatus ocellatus* (Fig 3A and 4D). The distal convoluted tubule of the lizard *Uromastyx acanthinura* primarily reabsorbs solute and was relatively impervious to water. Although reptiles' distal cell type doesn't seem to be able to create diluted urine (Dantzler, 1989), there were still a lot of morphological and functional differences between different species of reptiles. Na^+ and $C1^-$ were likely reabsorbed in the distal segment of amphibians without the support of water's osmotic effects (Nishimura and Imai, 1982). Similar to the distal nephron of *Cynops pyrrhogaster* and *Rana catesbeiana*, the distal tubule of *Rana cancrivora* appears to be an important site for the resorption of filtrates (Uchiyama *et al.,* 1990; Long, 1973; Hoshi *et al.,* 1981). It might also have a role in regulating body water.

In the kidney of *Chalcides ocellatus ocellatus* there was a connecting tubule that separates the distal and collecting tubules and was made up of two different types of cells; the



(A) longitudinal section stained with H&E showing the connecting tubule (CnT), the mucous cells (MuC), the non-mucous cells (NMuC), the intermediate segment (IS) and the collecting tubule (CT); (B) Transimission electron micrograph from the connecting tubule illustrating the non-mucous cells (NMuC) having their cytoplasmic protrusions (CP), the mucous cell (MuC) including the canal and the lumen of the connecting tubule (Lu); (C) transverse section stained with H&E showing the proximal convoluted tubule (PCT) and the collecting duct (CD) surrounded by a thin layer of smooth muscles (SM).

Fig 5: Photomicrographs of *Chalcides ocellatus ocellatus* kidney.

non-mucous cells, which have a pale cytoplasm and dense basal nuclei and the pink cells, which are mucous cells that was scattered between the other cells (Fig 5A). At the TEM level, the mucous cells have an intracellular canal through which they can secrete their mucus into the tubule lumen. The non-mucous cells differ from the mucous cells in that they possess a basal nucleus, were rich in mitochondria and were distinguished by the presence of protrusions of the apical cytoplasm containing mitochondria. Mucous cells also have a slightly basal nucleus and were rich in lysosomes (Fig 5B). Numerous clear cells (non-mucous cells) and certain mucous cells can be noticed in *Uromastyx acanthinura's* connecting tubule. This tubule may play a significant role in the absorption of water. A significant portion of the filtered fluid was reabsorbed in some amphibian and reptile species via the connecting tubule and collecting duct (Dantzler, 2016). Mammals, birds, some amphibians and reptiles' renal tubule reabsorption of fluid was influenced by their necessity to retain water (Dantzler, 2016). It was commonly accepted that variations in the permeability of the distal sections of the nephrons and collecting duct to water were the cause of variations in the osmolality of the ureteral urine of reptiles (Talmatamar *et al.,* 2020).

The collecting duct system comprises collecting tubules and collecting ducts. The collecting tubule was the first part of the collecting duct system that differs in structure in the kidney of each species. In the kidney of *Ptychadena mascarenensis*, the collecting tubule was heterocellular and characterized by the presence of two cell types; principal cells, which were darkly stained and intercalated cells, which were lightly stained and rich in mitochondria, which appeared in TEM. The apical surface of intercalated cells bears a few microvilli. Both cell types of the collecting tubule were cuboidal to pyramidal in shape, with basal spherical nuclei. Some of these nuclei were irregular in shape (Fig 3B). Histologically, there was no difference in structure between the collecting tubule and the collecting duct but the collecting duct was longer. Both were lined by cuboidal epithelial cells based on the basement membrane (Fig 2A and B). According to Madsen *et al.* (1988), the principal cells in the collecting duct were in control of potassium secretion while the intercalated cells were responsible for hydrogen ion secretion.

In the kidney of *Chalcides ocellatus ocellatus*, the collecting tubule was made up of low columnar epithelial cells based on the basement membrane (Fig 3D). The collecting duct was lined with cuboidal epithelial cells and was enclosed by a thin layer of smooth muscles (Fig 5C). According to Talmatamar *et al.* (2020), a substantial layer of smooth muscles surrounds the collecting duct epithelium in the kidney of *Uromastyx acanthinura*. In *Uromastyx acanthinura*, the importance of the muscular sheath around the collecting duct may be indicative of neurological control of the renal filtration rate. According to Thongboon *et al.* (2017), the function of the muscle layer may be connected to the systolic action that pushes the filtrate down the nephron. Tsuneki *et al.* (1984) hypothesized that the contraction of smooth muscles around the collecting duct caused by neural stimulation could raise intratubular pressure and impact the rate of renal filtration. The bursting of peritubular capillaries brought on by the constriction of these muscles may also have an impact on the circulatory activity of tubule cells.

CONCLUSION

All these kidney structural traits that affect body water balance could be viewed as adaptive mechanisms that allow *Chalcides ocellatus ocellatus* to survive dehydration in arid environments while *Ptychadena mascarenensis* provides adequate systems for living in freshwater.

Declaration section

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Availability of data and material

All the datasets generated or analyzed during this study are included in this published article.

Authors' contributions

All authors contributed equally to the study.

Ethical approval

The study was approved by the ethical committee at the Faculty of Science, Helwan University (approval number HU-IACUC/Z/TG1121-19) and according to the National Institutes of Health guide for the care and use of laboratory animals.

Consent to participate

All authors agreed to participate in this study.

Consent for publication

All authors agreed to publish this data.

Conflict of interest

The author(s) declare that they have no conflict of interest regarding the content of this article.

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