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Original Article

Microanatomical structure of the dog-faced water snake (*Cerberus rynchops*) from Thailand: A functional unit of the kidney

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Abstract Dog-faced water snake (*Cerberus rynchops*) is a well-known species inhabiting along coastal area in particular mangrove forests in Thailand. To delineate microstructure of kidney, mammalian species has been mostly used as an animal model, but its structures in snakes are still much less discussed in any literature. For providing evolutionary perspectives on reptilian kidney and comprehensive knowledge of vertebrate kidney, here we aimed to examine microstructure of kidney and uriniferous tubule in the dog-faced water snake using histological and histochemical techniques, which might be able to use as a new animal model for observing anatomy and physiology of squamates. Based on histological sections, several uriniferous tubules in the kidney were observed with differential characterizations. Each uriniferous tubule consists of a urine-forming nephron and a collecting duct. A nephron can be further subdivided into a renal corpuscle (a glomerulus and a Bowman's capsule) and a renal tubule. Three distinguishable parts of convoluted tubule segments under light microscopic level included proximal, intermediate and distal convoluted tubules. All segments of renal tubules were comprised of simple cuboidal epithelium between lumen and basal lamina; however, each tubular segment exhibited some histologically distinct features. The enlarged tubular size (approximately 50 μm in diameter) and the presence of apical brush border with clear PAS staining were detected in proximal convoluted tubule; whereas, the apical brush border could not be found in the intermediate tubule. In addition, collecting duct and metanephric duct (ureter) exhibited the clear distinct epithelium structure, compared to other renal tubular segments. Taken together, the histological study of kidney in dog-faced water snakes not only provides an insight into snakes/reptilian metanephros-derived urinary system but also will potentially lead to more understanding of ultrastructure and physiology of snakes in estuaries of Thailand.

Keywords: estuarine snake, histology, renal structure, Thailand

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บทความต้นฉบับ

จุลกายวิภาคของท่อยูรินิเฟอรัสในงูปากกว้างน้ำเค็ม (*Cerberus rynchops*) จากประเทศไทย: หน่วยพื้นฐานของไต

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บทคัดย่อ งูปากกว้างน้ำเค็ม (*Cerberus rynchops*) จัดเป็นสิ่งมีชีวิตที่รู้จักกันดีในบริเวณชายฝั่ง โดยเฉพาะป่าชายเลนของประเทศไทย การอธิบายถึงจุลกายวิภาคของไตนิยมใช้สัตว์เลี้ยงลูกด้วยน้ำนมเป็นสัตว์ทดลอง แต่อย่างไรก็ตามการศึกษาโครงสร้างดังกล่าวในงูมีน้อยมากจากการทบทวนเอกสารอ้างอิง ดังนั้นในการศึกษานี้เพื่อให้ได้ความรู้ทางด้านวิวัฒนาการของไตของสัตว์เลื้อยคลานและองค์ความรู้โดยรวมของไตในสัตว์มีกระดูกสันหลัง การศึกษานี้ได้ศึกษาอธิบายถึงจุลกายวิภาคไตและท่อยูรินิเฟอรัสของงูปากกว้างน้ำเค็ม ด้วยเทคนิคทางด้านมิญชวิทยาและมิญชเคมี ที่ต่อไปอาจใช้งูชนิดนี้เป็นสัตว์ทดลองชนิดใหม่เพื่อศึกษากายวิภาคและสรรีวิทยาของสัตว์เลื้อยคลานกลุ่ม squamates จากการเตรียมเนื้อเยื่อเพื่อศึกษาทางมิญชวิทยา พบท่อยูรินิเฟอรัสหลายท่อในเนื้อเยื่อไตที่มีความแตกต่างกันทางด้านจุลกายวิภาค ท่อยูรินิเฟอรัสแต่ละท่อประกอบด้วยเนฟรอนและท่อไตรวม ส่วนเนฟรอนประกอบด้วยรีนัลคอร์พัสเคิล (โกลเมอรูลัสและโบว์แมนส์แคบซูล) และท่อไตที่แบ่งเป็นท่อไตส่วนต้น ท่อไตส่วนกลาง และท่อไตส่วนท้าย ภายใต้กล้องจุลทรรศน์แบบใช้แสง ทุกท่อไตมีเยื่อบุผิวแบบลูกบาศก์ชั้นเดียว แต่มีโครงสร้างทางจุลกายวิภาคบางอย่างแตกต่างกัน ท่อไตส่วนต้นมีขนาดใหญ่ประมาณ 50 ไมโครเมตรและมีบรัชบอร์เดอร์ ทำปฏิกิริยากับพีเอเอช ท่อไตส่วนกลางไม่พบบรัชบอร์เดอร์ นอกจากนั้นยังพบว่าท่อไตรวมและท่อเมโซเนฟฟริคมีโครงสร้างเยื่อบุผิวที่แตกต่างชัดเจนจากท่อไตส่วนอื่น เมื่อเทียบกับท่อไต ดังนั้น ในงานวิจัยทางมิญชวิทยานี้ได้ทำให้ได้รับองค์ความรู้เกี่ยวกับจุลกายวิภาคของระบบขับถ่ายของงูและสัตว์เลื้อยคลานที่พัฒนามาจากเมตาเนฟรอสซึ่งเป็นข้อมูลสำคัญ สำหรับการการศึกษาโครงสร้างละเอียด และสรีรวิทยาของงูบริเวณชะวากทะเลในประเทศไทย

คำสำคัญ งูปากแม่น้ำ, มิญชวิทยา, โครงสร้างไต, ประเทศไทย

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Introduction

The reptile kidney is intriguing and worth studying because it could be applied in several types of research relating to physiology and histopathology as well as evolutionary origin. As well, anatomical and histological evidences of the kidney in the reptile are paired and closely located to the gonadal structure in the abdominal cavity (Zuasti et al., 1987; Kent and Carr, 2001), in which the ontogenic kidney during the reptile embryonic development was described as the mesonephros of the larval stage. Then, it transformed into the metanephros of the adult stage (Edward, 1998). Although the complexity during differentiation of the kidney has widely been reported, it is generally composed of several uriniferous tubules, as a functional unit of the kidney. Each tubule contained the nephron and collecting duct. Typically, the nephron also known as nephric tubule is comprised of the renal (Bowman's) capsulelong and ciliated proximal convoluted tubule, a ciliated intermediated segment, and a short distal convoluted tubule. In reptiles, segment of intermediate tubule is relatively short and lacks of loop of Henle (reptilian-type nephron). In contrast, the elongated intermediate segment with loop of Henle is present in mammals (mammalian-type nephron). Additionally, change in glomerular filtration rate (GFR) may involve adjustments in a relative proportion of reptilian- and mammalian-type nephrons (Bishof, 1959; Dantzler and Bradshaw, 2009; Bradshaw and Bradshaw, 2013; Wyneken, 2013). Unlike the mammalian and amphibian, various reptile-specific components in the renal corpuscle including a majority of the glomeruli and Bowman's capsule are detected. as shown in some reptile kidney system such as *Testudoaracea* (Zuasti et al., 1987) and *Acanthodactylus boskianus* (Yari and Gharzi, 2013).

The dog-faced water snake (*Cerberus rynchops*) is a well-known estuarine snake and commonly found in coastal areas of Southeast Asia such as Myanmar, Malaysian Peninsulas and Thailand. In recent years, this snake has been attractively examined in several disciplines including ecology, population structure and genetic diversity (Lv et al., 2015). Because of its high abundance in nature and sufficient preliminary data on its biology, this species of snake might probably be a great model to resolve some evolutionary mystery, comparing to other available data of reptilians and vertebrates. In particular, knowledge regarding renal anatomy and physiology of estuarine snakes is still missing. At this point, one query is how this special kind of snakes can inhabit a harsh environment of mangrove environment with the punctuality of water/osmolarity and salinity. To better understand the uniqueness and common features to other vertebrate kidneys, insightful information on the structure of the snake's kidney becomes essential. We hereby described the micro-anatomical structure of the uriniferous tubule of *C. rynchops*, obtained from Estuary Paknam Pranburi, Thailand using the histological and histochemical techniques, which conceivably provide more understanding on how the snakes can cope with estuarine environment.

Materials and methods

Carcasses of dead female, *Cerberus rynchops* during adult stage (n = 5, the snout-vent length 74±4.06 cm) were donated from fishmongers at two stations (N 12°24'15.8" / E 099°58'25.6" 2 and N 12°24'21.6" / E 099°58'37.1"). The kidney tissues were detached from middle line of the abdominal region. Each tissue was collected from two areas (anterior and posterior kidneys) and then was fixed in 4% paraformaldehyde at 4 ºC (about 24 hours) under the histological techniques.

Following Presnell and Schreibman (2013) and Suvarna et al. (2013), fixed kidneys were processed via standard histological techniques, and then paraffin blocks were cut at 4 μm thick. All sections were stained with Harris's hematoxylin and eosin (H&E) as well as histochemically stained with Periodic Acid Schiff (PAS) to detect the glycoprotein and Masson's trichrome (MT) to identify the connective tissue and fibers. Evidence of the histological structures of the kidney tissues was finally observed and photomicrographed under a light microscope equipped with a camera for photography (TE2000-Ua).

Results and discussion

We clearly demonstrated the kidney microanatomy of the *C. rynchops* based on the histological and histochemical techniques (as in Figures 1A-1B). Regarding the metanephric kidney, it was paired and slender organs which were retroperitoneal and located closely in the reproductive system (Data not shown), as likely reported in *Sceloporus cyanogenys* (Davis et al., 2005). It was also crossly covered by a thin capsule with inserting smooth muscle as greenish color (Figure 1C; MT method). Although the cross section from two regions (anterior and posterior regions) could not be clearly identified into cortex and medulla, it was similarly composed of several uriniferous tubules (Figures 1A-1B). Each uriniferous tubule was composed of nephron and collecting duct (Figures 1A-1B). This similarly described other studied reptiles (Edward, 1998), for example, *Crocodylusacutus* kidneys (Davis and Schmidt-Nielsen, 1976) and *Caiman crocodilusyacare* (Jin et al., 1993).

Our examination revealed that the renal corpuscle of *C. rynchops* was small in size (about 70-80 μm in diameter) and formed by a simpler system of a filtration unit (capillary loop), as also called a glomerulus; however, the structure of the renal corpuscle and glomerulus was rarely found in the kidney tissue. This was similarly reported in some reptiles (Davis et al., 2005; Zarnik, 1910). A possible reason for the structural modification and the presence of a small and few renal corpuscles might relate to the adaptation to prevent water loss and to control the excretion of nitrogenous compounds with low glomerular infiltration under extreme environment such as estuarine condition, as suggested by several investigators (Pequeux et al., 1983; Dantzler, 2005; Allam and EA Eleneen, 2013).

As a result, the glomerulus was approximately 50 μm in diameter and clearly defined by PAS and MT staining into two features, which were urinary and vascular poles (Figures 1D-1E). The glomerular filtration membrane was sharp and composed of various cell compositions such as the podocyte and red blood cell (Figures 1D-1E), similar to those arrangement in *Alligator mississippiensis* (Moore et al., 2009).

In terms of podocyte, it was an elongated cell with an oval nucleus and basophilic cytoplasm, whereas the oval shape of the red blood cell in the glomerular capillaries was seen. This was also observed in some studied snakes (such as *Eryxjaculus*, *Psammophissibilans* and *Echispyramidium*) from Egyptian area (Moore et al., 2009). The important role of the podocyte of the snake is still unclear (Allam and Eleneen, 2013). It was suggested that podocytes in snake bore several microvilli and large bundle of microfilaments (Peek and McMillan, 1979), and this might probably indicate the function of podocyte in the regulation of glomerular filtration rate. The glomerulus was also surrounded by double layers of Bowman's capsule including parietal and visceral layers (or glomerular epithelium) (Figures 1D-1E).

Microscopically, the histological structure of the renal tubule connected to the renal corpuscles at the urinary pole. This tubule was classified into three convoluted tubule segments (Figures 1F-1G). The first segment of proximal convoluted tubules has the greater diameter, approximately 50 μm in diameter and a small cavity (about 5 μm) (Figure 1G). It was lined by a simple cuboidal epithelium. In the same way, a good development of the apical structure in both brush border and cytoplasm in the luminal epithelium was positively reacted in PAS as pinkness (Figures 1F-1G), similar to previous observations in some reptiles (Ventura et al., 1989; Jin et al., 1993).

In transverse sections of *C. rynchops* proximal convoluted tubule, the cuboidal epithelial cell containing spherical nucleus was also seen, similar to those observed in *Crocodylus acutus* (Davis and Schmidt-Nielson, 1867) and some terrestrial lizards (Roberts and Schmidt-Nielsen, 1966). The proximal convoluted tubule was basally located with strong reddish cytoplasm (PAS staining method) , indicating the presence of glycoprotein (Figure 1F). In previous studies, it was suggested that this glycoprotein may indicate the abundance of the mitochondria in the cytoplasm (Soares and Fava-De Moraes, 1984; Roberts and Schmidt-Nielsen, 1966). This implication plays role in the ion-transporting cell throughout the uptake of an isotonic absorbate and the importance of the exchange of fluids (Farias et al., 1998).

Sections of the intermediate convoluted tubule was lined in similarly to the first segment of proximal convoluted tubule, but the size of this tubule was decreased about 20 μm diameter (Figure 1F). Also, the apical brush border was absent (Figure 1F). The second distal convoluted tubule was as similarly lined with the intermediate convoluted tubule, but the size of this segment was increased about 30 μm in diameter (Figure 1F). The brush border was detected and the apical cytoplasm of the cuboidal cell exhibit a faint pinkness staining (PAS staining) , compared to the proximal convoluted tubule (Figures 1F-1G).

An explanation of this unique feature is still unknown, and it is possible that brush border in this distal segment might involve in the re-absorption processes (Danzler and Holmes, 1974).

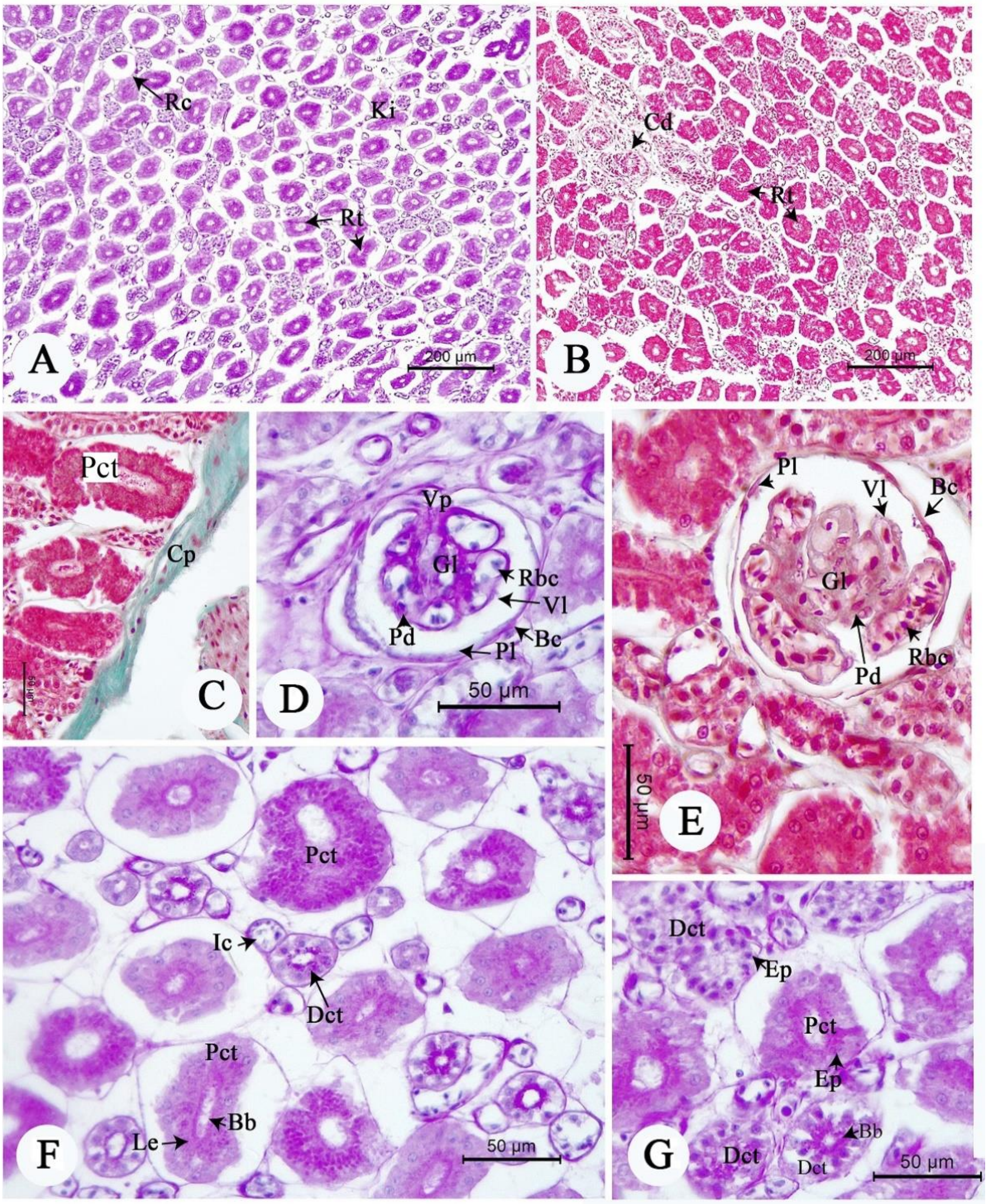

Figure 1. Microanatomy of the uriniferous tubules in *Cerberus rynchops* kidney (Ki) sections (A-H). Labels: Bb = brush border, Bc = Bowman's capsule, Cd = collecting duct, Cp = capsule, Dct = distal convoluted tubule, Ep = epithelium, Gl = glomerulus, Ic = Intermediate convoluted tubule, Le = luminal epithelium of the cytoplasm, Pct = proximal convoluted tubules, Pd = podocyte, Pl = parietal layer, Rbc = red blood cell, Rc = renal corpuscle, Rt = renal tubules, Vl = visceral layer, Vp = vascular pole.

The histological structure of the collecting duct system was continuously connected to the second distal convoluted tubule. It is composed of collecting tubules and collecting ducts. Cell of the collecting duct had a tall columnar epithelium with central to basal nuclei (Figure 2A) and was similar to *Cyrtopodionscabrum* (Al-Shuwaili, 2015). Guzsal (1970) suggested that the role of epithelial cells in the collecting duct is the production of mucin to support the kidney functions. This role is also recorded in other vertebrates such as fishes, reptiles, and birds (Al-Shuwaili, 2015; Guzsal 1970). The collecting duct was surrounded by the layer of mucularis (as greenish with MT reaction) with inserting to several capillaries (Figure 2A). We proposed that the role of the muscularis layer could possibly be related to the movement of the collecting duct, which needs to be further studied. After this duct transformed into a larger duct before entering the metanephric duct (Figures 2B-2D), the metanephric duct of the snake was well developed as the enlarged efferent duct. The epithelial layer was covered with pseudostratified columnar epithelium (Figures 2C-2D), as likely seen in some vertebrates (Rosenfeld et al., 2000).

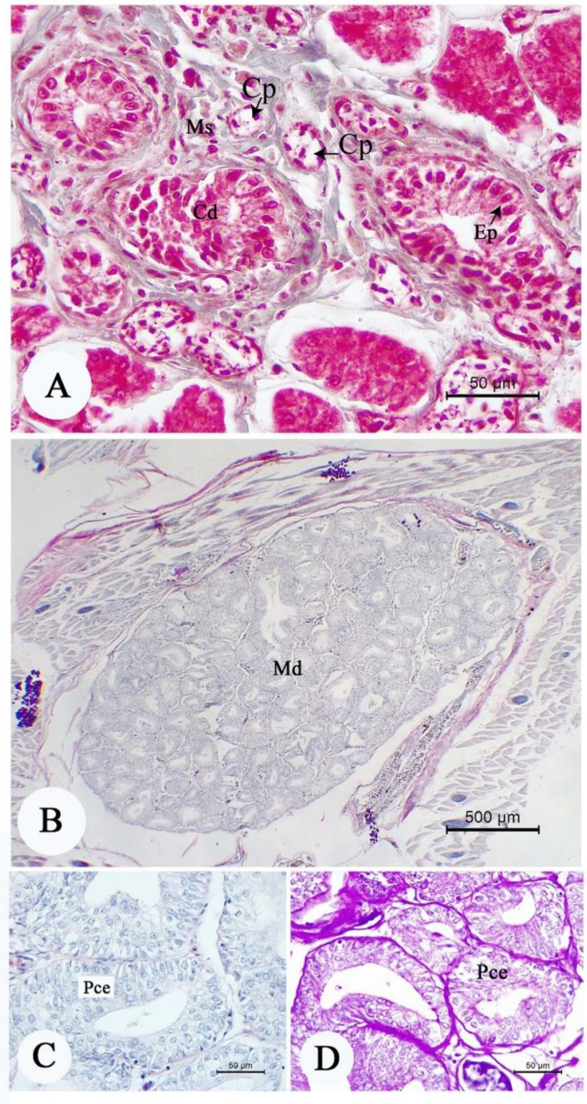

Figure 2. Microanatomy of the collecting duct (Cd) and metanephric duct (Md) in *Cerberus rynchops* kidney sections (A-D) Labels: Cp = capillaries, Ep = epithelium, Ms = muscularis layer, Pce = pseudostratified columnar epithelium

Conclusions

The histological structure regarding the kidney together with uriniferous unit (the nephron and collecting duct) of *C. rynchops*are here is considered as the first descriptions of microscopic examination in estuarine snake. We suggested that the presence of a few renal corpuscles in the kidney of *C. rynchopsare* is a unique feature and probably relates to its adaptation to estuarine condition. The findings and knowledge from this our study would be beneficial for further studies such as in physiology and histopathology as well as evolutionary trends of squamates.

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