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Structure Organization of Urinary System in the Yellow Spotted Mountain Newts (Salamandridae: *Neurergus microspilotus*)

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Abstract This study deals with the histomorphology of the mesonephros in male and female *Neurergus microspilotus*. The slender and narrow kidneys are positioned in the retro peritoneal position up against the ventral aspect of vertebral column and may extend the length from the esophagus-stomach junction to cloaca. The kidney in both sexes is composed of sexual (anterior) and pelvic (posterior) parts. The duct of sexual kidney is a narrow duct which is lying alongside its lateral edge. In the female, it is connected to the ureters and then the duct of definitive kidney. Before entering the cloaca, two ureters are joined together and open to the apex of the cloaca. In the male, after entering the sexual kidney, the sperm leave the testis through efferent ducts, then these ducts join together and eventually form Bidder's duct. The Bidder's duct joins the Bowman's capsule of the nephrons in the sexual kidney and the nephrons make collecting ducts which are filled with both sperm and urine. After leaving the kidney, all the collecting ducts are connected to the Wolffian duct. Wolffian duct joins the ureters (merge from definitive kidney) just before entering the cloaca. Based on serial paraffin sections, nephrons consist of a filtration unit, the Malpighian corpuscle, and a renal tubule, which can be divided into 4 morphologically distinct segments: proximal tubule (first and second segment), distal tubule, and collecting tubule. Collecting tubules merge and form a branch system that opens into collecting ducts.

Keywords *Neurergus microspilotus*, sexual kidney, pelvic kidney, ureters

1. Introduction

Amphibians have mesonephric kidneys. The embryonic kidney of amphibians and fish is known as the pronephros, head kidney, or vorniere, while their adult kidney is known as a mesonephros, Wolffian body, or urniere. In animals, the mesonephros is the terminal kidney, and the final organ is very complex, containing a large number of nephrons. Most of nephrons have internal glomerulus and in some instances, particularly in anterior mesonephric tubules, also link to coelom via ciliated tubules called peritoneal funnels which are referred to nephrostomes (Wright, 2001; Richard, 1929). Salamanders possess paired kidneys that can generally be divided into two parts: the anterior part or sexual kidney and the posterior part termed the definitive kidney (Baker and Taylor, 1964) or the pelvic kidney (Spengel, 1876; Siegel *et al*., 2010). There is a differentiation in the structure and function of two parts of the kidney. In the male, the testes are connected to the Wolffian ducts through modified nephrons which are located in the sexual kidney and are responsible for the transport of sperm with variable morphologies (Williams *et al*., 1984). The definitive kidney is involved in urine transport from the nephrons to the cloaca and the production of the sexual secretion in some taxa (Adams, 1940; Norris, 1987; Siegel *et al*., 2010). In the female, the two parts of the kidney are distinguishable, although the sexual kidney has no connection with the gonads (Francis, 1934).

The functional unit of the kidney is the nephron or kidney tubule (Saxen, 1987). Each nephron consists of a renal corpuscle and a convoluted tubule which has three parts, with variable length in different species

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(Mobjerg *et al*., 2004; Kriz and Kaissling, 2008). Nephrons empty into collecting ducts that exit the kidney and connect with the Wolffian duct or the cloaca. In some salamanders, the collecting ducts bend caudally during development (Rodgers and Risley, 1938) and either anastomose and enter the cloaca (in the Plethodontidae and some Salamandridae) (Francis, 1934; Baker, 1965; Strickland, 1966), anastomose and enter Wolffian ducts (in the Ambystomatidae, Amphiumidae, and some Salamandridae) (Baker and Taylor, 1964; Baker, 1965), or enter the cloaca individually (in the Cryptobranchidae and Hynobiidae) (Ratcliff, 1965).

Less information is available for the urodele urinary system compared with anurans (Jobling, 2011). The aim of the present work is to establish baseline information on kidney and ureter histology and anatomy for female and male *N. microspilotus* during the breeding season and provide data for a possible comparative analysis with closely related species.

2. Materials and Methods

The present study on the structural organization of the urinary system in the yellow spotted mountain newt was conducted in conjunction with a conservation management plan funded by the Mohammad Bin Zayed Species Conservation Fund. Part of this plan included development of a captive breeding facility (CBF) at Razi University, Kermanshah, Iran. The goal of the CBF is to stock and increase the species' population size from different breeding streams in order to ensure their long term survival, to supplement wild populations to a self-sustaining level, or to re-establish this species in parts of its former range (Sharifi and Vaissi, 2013). Two male and two female *N. microspilotus* were collected from the CBF. These newts belong to a stock built up from two males and four females originally collected from Kavat Stream in northern Kermanshah in western Iran (34˚53' N; 46˚31' E). The animal treatment and care in this experiment followed guidelines provided by AZA Amphibian Husbandry Resource Guide (Poole, 2012).

All animals under resting condition had the average total length of 192.35 ± 10.20 mm. The total length was measured as the distance from the tip of the snout to the end of the tail. Measurements of the kidney were taken by digital Vernier Calliper. Measurements are expressed as mean ± standard error. Then urogenital tracts were removed from the body cavity and fixed with 10% neutral buffered formalin, dehydrated in a series of ethanol treatments, starting from the 70% storing solution, cleared in xylene, embedded in paraffin, and serially sectioned at 5–7 µm with a rotary microtome. Staining was carried out with Hematoxylin-Eosin (HE) (Luna, 1968). Sections were observed with an Olympus microscope (Leica Galen III) and were photographed with a digital camera (Leica with Dinocapture 2).

3. Results

The kidneys in male and female *N. microspilotus* lie in the retro peritoneal position up against the ventral aspect of vertebral column and can extend from the esophagus-stomach junction to cloaca. Kidneys are slender and narrow anteriorly. The length averages 13 ± 0.64 mm; width averages 2.3 ± 0.43 mm. The kidney can be divided into two parts: the anterior narrow part or sexual kidney and the posterior thick part or definitive (pelvic) kidney. The duct of the sexual kidney is a fine longitudinal canal lying alongside its lateral edge (Figure 1). In the female, this duct is joined to the ureters and the duct of the definitive kidney. The ureters pass through the mesentery supporting the oviduct. Before entering the cloaca, ureters join together and make one duct which opens into the cloaca at the apex of the small papilla just dorsal to the

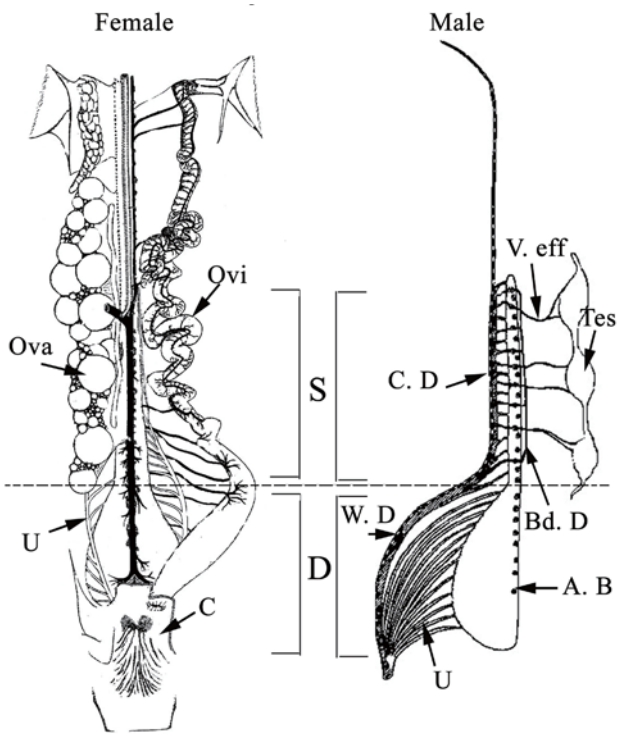

Figure 1 Diagrammatic representation of male (Left) and female (Right) urogenital system. S) sexual kidney, D) definitive kidney, ova) ovary, ovi) oviduct, U) ureter, C) cloaca, Tes) testis, V.eff.) vasa efferentia, Bd.D) Bidder's duct, C.D.) collecting duct, W.D.) Wolffian duct, A.B) adrenal body.

opening of Muller's duct (oviduct). It remains entirely separated from Muller's duct along its length (Figure 2 A). The sexual kidney in male *N. microspilotus* is associated with the gonad and the vasa efferentia connects to the Wolffian duct directly, while the sperms actually pass through its tubules. Firstly, although the tubules are small as they leaving the kidney, they expand very rapidly and become large and thick walled in the male. Secondly, the urogenital duct draining the sexual kidney is joined by the purely urinary ducts from the definitive kidney near the cloaca (Figure 2 B). The *N. microspilotus* kidney is mesonephros and composed of nephrons, its functional unit. A typical nephron consists of distinct regions: a renal corpuscle, proximal and distal tubules. The distal tubules connect to small collecting tubules, which join together and make larger collecting ducts that empty into ureters.

Renal corpuscle (glomerulus within Bowman's capsule): Each glomerulus contains a compact mass of mesangial cells (small cells with large nuclei) at its center. The mass appears basophilic because of the relatively high concentration of nuclear material. A layer of podocytes, with large round or oval nuclei, covers the surface of the glomerular capillaries, forming the glomerular (visceral) layer of Bowman's capsule. The capsular (parietal) layer of Bowman's capsule consists of a simple squamous epithelium (Figure 3 A).

Proximal convoluted tubule (PCT): First segment (P1): Tall cuboidal cells with large spherical, pale-staining, basally located nuclei; and short brush border (compact microvilli) (Figure 3 B). Second segment (P2): Simple columnar cells with oval to rounded, centrally located nuclei; a long and dense apical brush border; and intensely eosinophilic cytoplasm (Figure 3 C).

Distal convoluted tubule (DCT): simple cuboidal epithelium with round, centrally located nuclei with less brush border and stain paler than PCT (Figure 3 D).

Collecting tubules: simple cuboidal ciliated cells with basally located nuclei, and a distinct lateral border among these cells (Figure 3 C).

In the female, the nephron structure in the sexual and definitive kidney is the same and it is similar to those of the male. The sperm leave the testis through efferent ducts (vasa efferentia), which is 4–5 fine ducts after entering the sexual kidney. The vasa efferent come together and form Bidder's ducts. The duct is covered with tall columnar epithelium. The cells are filled with acidophilic granules (secretory) (Figure 4). Bidder's duct is joining the Bowman's capsule of nephrons. Nephrons make collecting ducts which are filled with both sperm and urine. Collecting ducts are lined with simple low cuboidal epithelium. After exiting the kidney, all collecting ducts connect to the Wolffian duct which is lined by pseudo-stratified columnar epithelium. Myoepithelial cells are present beneath the epithelium, and a thick layer of circular muscle bundle forms tunica muscularis. A single layer of mesothelium is present at tunica serosa (Figure 5). Wolffian duct joins the ureters just before entering the cloaca.

The ureters are parallel ducts after exiting the definitive kidney (Figure 1). They are small tubules with a ciliated simple cuboidal epithelium. Myoepithelial cells are seen beneath the epithelium (Figure 6). There is no renal pelvis or urinary bladder in *N. microspilotus*.

4. Discussion

Similar to *Ambystoma maculatum* (Siegel *et al*., 2010), *Triton* (Gray, 1932) and *Salamandra salamandra* (Francis, 1934), the kidneys in *N. microspilotus* are mesonephric, paired and symmetrical. Each is narrow and ribbon-like anteriorly and becomes wider and thicker posteriorly. In the male, the former portion is called the sexual kidney on account of its association with the gonad; but in the female, sexual kidney is named only since it has no connection with the female gonad. Like all salamanders, except Plethodontidae (Strickland, 1966; Williams *et al*., 1984), Rhyacotritonidae, and Sirenidae (Willett, 1965), in *N. microspilotus* multiple vasa efferentia connect together and make the Bidder's duct, which join to the efferent epidydymal ducts. Bidder's duct differs from the testicular longitudinal canal that has also been termed as the longitudinal duct in some studies. The afferent epididymal ducts empty into the renal corpuscle in male *N. microspilotus* like all salamanders except the Eurycinae, Rhyacotritonidae, and Sirenidae (Baker and Taylor, 1964; Williams *et al*., 1984). Like many amphibian species (Mobjerg *et al*., 1998; Mobjerg *et al*., 2004), the morphology of the nephron in *N. microspilotus* consists of a renal corpuscle, proximal tubule, distal tubule, and collecting tubules and ducts. The renal corpuscle in *N. microspilotus*, like that of *Ambystoma maculatum* (Siegel *et al*., 2010), is composed of the Bowman's capsule and a fine network of glomerular capillaries. The Bowman's capsule has a parietal epithelium that is continuous with the visceral epithelium; the visceral epithelium is in close association with the capillaries of the glomerulus. The proximal tubule can be divided cytologically into two distinct regions: first segment (P1) and second segment (P2). In comparison with the proximal tubule of

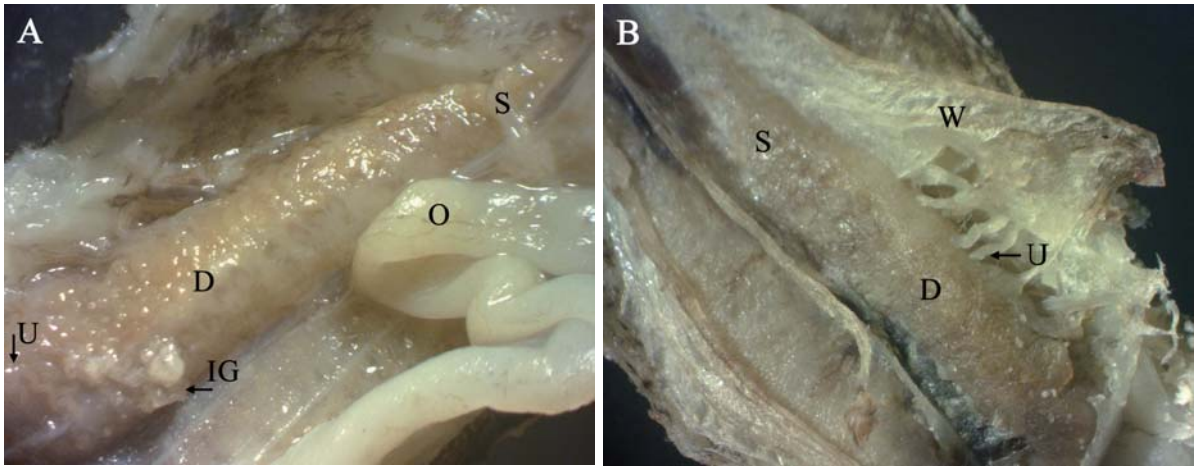

Figure 2 A) Gross anatomy of the kidney in a female *N. microspilotus*. The kidney is divided into two parts, the narrow sexual (S) and the thick definitive kidney (D). The inter renal glands (IG) are seen in the ventral surface of the kidney near the median line. O: Oviduct, U: Ureters. B) Ventral view of the kidney in male *N. microspilotus*. S: Sexual kidney, D: definitive kidney, U: Ureters, W: Wolffian duct. *: the junction of Wolffian duct and ureters just before entering the cloaca.

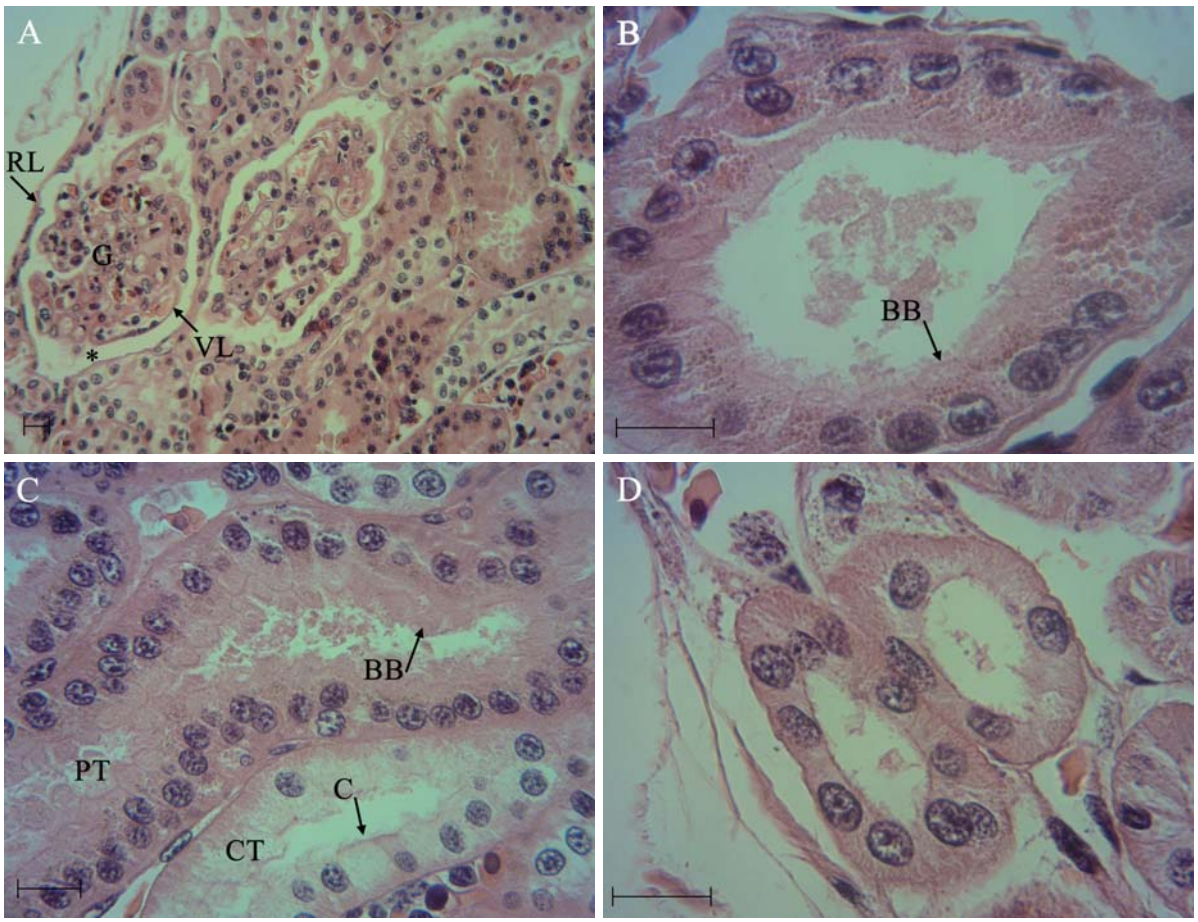

Figure 3 A) Photomicrograph of the kidney of *N. microspilotus*. Renal corpuscle showing the glomerulus (G) and Bowman's space (*). PL: Parietal Layer, VL: Visceral Layer, Arrowhead: Mesengial cell. B) Photomicrograph of the first segment of the proximal convoluted tubule. Tall cuboidal cells with compact short microvilli, Brush Border (BB) can be seen. C) Photomicrograph of the second part of proximal convoluted tubule (PT), simple tall columnar cell with basal round nuclei and long brush border (BB) and acidophilic cells are seen. In the collecting tubules (CT) the epithelial cell is less acidophilic and the lateral border of the cells is evident. Cilia can be seen in these tubules. D) Photomicrograph of distal convoluted tubules. The epithelium is composed of low cuboidal cells with round nuclei. Scattered microvilli are present. Bar: 20 μm.

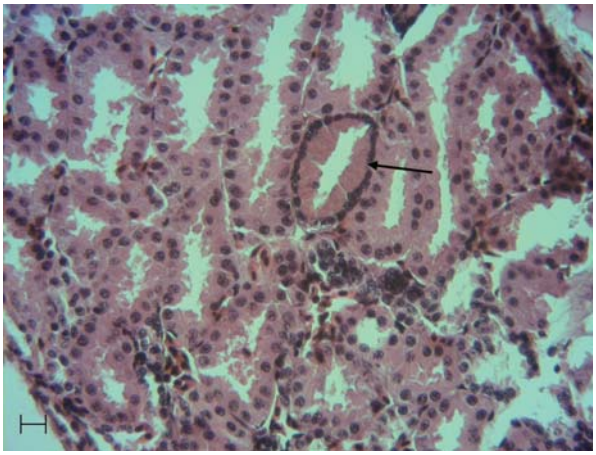

Figure 4 Bidder's duct (arrow) between renal tubules in the sexual kidney in male *N. microspilotus*. This duct is covered with tall columnar acidophilic cells with basal nuclei. Bar: 20 µm.

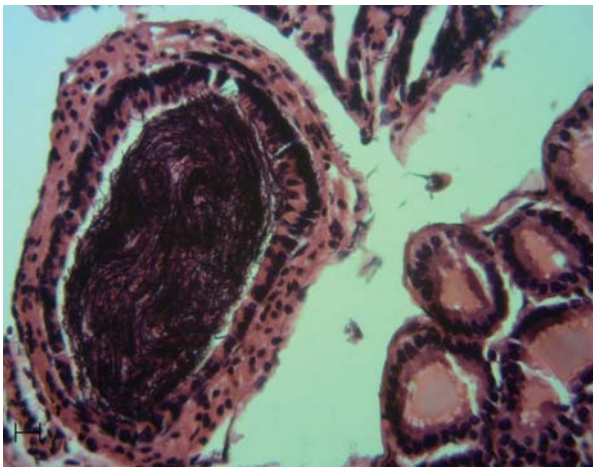

Figure 5 Photomicrograph of the Wolffian duct in male *N. microspilotus*. The duct is covered with psudostratified columnar epithelium. The circular muscle is present in tunica muscularis and a single squamus cell of mesothelium is present in the outermost layer. The Wolffian duct is filled with sperm and urine. Bar: 20 µm.

Ambystoma tigrinum, *Amphiuma means* (termed proximal segment), *Necturus maculosus*, *Ambystoma maculatum*, and *Triturus pyrrhogaster*, the second portion of the proximal tubule in *N. microspilotus* is indistinguishable. The apical border of P1 in *N. microspilotus* is covered entirely by microvilli, as it was reported by Clothier *et al*., (1978), Sakai and Kawahara (1983), and Maunsbach and Boulpaep (1984) for other species of amphibians.

Like many amphibian mesonephrose (Hentschel and Elger, 1989; Mobjerg *et al*., 1998), the distal tubule in *N. microspilotus* cannot be clearly subdivided into an "early" and "late" tubule because of differences in ultrastructure of the epithelial cells. The epithelium of the distal tubule in *N. microspilotus,* similar to *Ambystoma* maculatum

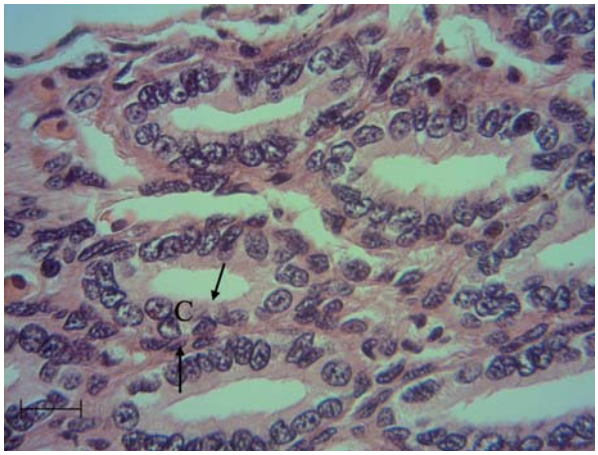

Figure 6 Photomicrograph of ureters in female *N. microspilotus*. These ducts are arranged in parallel and they are covered by simple cuboidal epithelium with cilia (C). Myoepithelial cells (arrow) are present beneath the epithelium. Bar: 20 µm.

(Siegel *et al*. 2010), is simple cuboidal with centrally positioned nuclei. Brush borders are common on the apical surface of the cells but not to the extent of the proximal tubules. Some variation clearly exists among the collecting ducts of salamandrids, ambystomatids, and plethodontids. Aron (1924) described the epithelium of the collecting ducts as nonciliated, simple, and tall columnar that contained numerous eosinophilic granules. Adam (1940) described the collecting ducts of *Notophthalmus viridescens* as similar to the Wolffian ducts with a similar seasonal cycle. Sakai and Kawahara (1983) provided the ultramicrograph of the collecting duct epithelium from another salamandrid, *T. pyrrhogaster*. Siegel *et al*., (2010) provided the ultrastructure of the collecting duct in *Ambystoma maculatum*. In this study, the collecting ducts in *N. microspilotus* are lined up by tall columnar cells with basal heterochromatic nuclei. Ciliated cells are scattered in between the apices of adjacent columnar principle cells. The collecting ducts and tubules are simple cuboidal ciliated, which are lightly eosinophilic with distinct lateral border of cells.

In *N. microspilotus,* the ureters in males and females are different, but in *Triton* (Gray, 1932) and *Salamandra salamandra* (Francis, 1934) they are the same in both sexes. In the female, the duct of the sexual kidney is a fine longitudinal canal which is joined to intervals by transverse tributaries from the tubules of the kidney. As it approaches, the Wolffian duct diverges its edge to some extent, and it passes through the mesentery supporting with oviduct, and then, just as the duct, it becomes folded over to the ventral side of the kidney just before entering the cloaca. The Wolffian duct receives tributaries from

the kidney and becomes correspondingly thicker. It opens into the cloaca at the apex of a small papilla just dorsal to the opening of oviduct and remains entirely separate from oviduct throughout its length. In the male, collecting ducts connected to Wolffian duct after leaving the kidney. The Wolffian duct is covered with psudostratified columnar epithelium. It joined to the ureters just before entering the cloaca. The ureters are small tubules with a ciliated simple cuboidal epithelium. Our results agree with Gray (1932) and Francis (1934). Obviously electron microscopic works are required to clarify the functional morphology of sexual kidney in this specious of salamander.

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