The Pelvic Kidney of Male *Ambystoma maculatum* (Amphibia, Urodela, Ambystomatidae) with Special Reference to the Sexual Collecting Ducts

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ABSTRACT This study details the gross and microscopic anatomy of the pelvic kidney in male *Ambystoma maculatum*. The nephron of male *Ambystoma maculatum* is divided into six distinct regions leading sequentially away from a renal corpuscle: (1) neck segment, which communicates with the coelomic cavity via a ventrally positioned pleuroperitoneal funnel, (2) proximal tubule, (3) intermediate segment, (4) distal tubule, (5) collecting tubule, and (6) collecting duct. The proximal tubule is divided into a vacuolated proximal region and a distal lysosomic region. The basal plasma membrane is modified into intertwining microvillus lamellae. The epithelium of the distal tubule varies little along its length and is demarcated by columns of mitochondria with their long axes oriented perpendicular to the basal lamina. The distal tubule possesses highly interdigitating microvillus lamellae from the lateral membranes and pronounced foot processes of the basal membrane that are not intertwined, but perpendicular to the basal lamina. The collecting tubule is lined by an epithelium with dark and light cells. Light cells are similar to those observed in the distal tuble except with less mitochondria and microvillus lamellae of the lateral and basal plasma membrane. Dark cells possess dark euchromatic nuclei and are filled with numerous small mitochondria. The epithelium of the neck segment, pleuroperitoneal funnel, and intermediate segment is composed entirely of ciliated cells with cilia protruding from only the central portion of the apical plasma membrane. The collecting duct is lined by a highly secretory epithelium that produces numerous membrane bound granules that stain positively for neutral carbohydrates and proteins. Apically positioned ciliated cells are intercalated between secretory cells. The collecting ducts anastomose caudally and unite with the Wolffian duct via a common collecting duct. The Wolffian duct is secretory, but not to the extent of the collecting duct, synthesizes neutral carbohydrates and proteins, and is also lined by apical ciliated cells intercalated between secretory cells. Although functional aspects associated with the morphological variation along the length of the proximal portions of the nephron have been investigated, the role of a highly secretory collecting duct has not. Historical data that implicated secretory activity concordant with mating activity, and similarity of structure and chemistry to sexual segments of the kidneys in other vertebrates, lead us to believe that the collecting duct functions as a secondary sexual organ in *Ambystoma maculatum*. J. Morphol. 000:000–000, 2010.   © 2010 Wiley-Liss, Inc.

KEY WORDS: urogenital; nephron; anatomy; histology; histochemistry; ultrastructure

INTRODUCTION

Salamanders possess paired kidneys that can generally be divided into cranial and caudal portions. The cranial portion of the male kidney has been termed the sexual (Francis, 1934; Rodgers and Risley, 1938) or genital kidney (Adams, 1940; Williams et al., 1984), in which the nephrons are modified for the transport of sperm from the vasa efferentia of the testes to the Wolffian ducts. In plethodontid salamanders, this cranial portion of the kidney is lost (Strickland, 1966), and only the nephrons forming the epididymal complex remain. Williams et al. (1984) and Aranzábal (2003, 2009) reviewed variation in cranial kidney structure.

The caudal portion of the male adult kidney, termed the definitive kidney (Baker and Taylor, 1964) or simply the pelvic kidney (Spengel, 1876), is involved in urine transport from the nephrons to the cloaca and the production of a sexual secretion in some taxa (Aron, 1924; (Norris, unpublished data; but see Norris, 1987)). In the Proteidae (Chase, 1923; Rosenquist and Baker, 1967) and Sirenidae (Willett, 1965), the proximal regions of the nephron communicate with the Wolffian duct via collecting ducts that exit the pelvic kidney laterally. These collecting ducts fuse with the Wolffian duct individually along its caudal length. This morphology is typical of the larval and female structure in salamanders (Francis, 1934; Rodgers

and Risley, 1938), and we term this the "simple condition."

In Ambystomatidae (Baker and Taylor, 1964), Amphiumidae (Baker, 1945), Cryptobranchidae (Ratcliff, 1965), Dicamptodontidae (de Marco, 1952), Hynobiidae (Yamagiwa, 1924), Plethodontidae (Strickland, 1966), and Salamandridae (Aron, 1924; Francis, 1934; Baker, 1965), the collecting ducts exit the kidney laterally and communicate with the cloaca. Each collecting duct communicates with the cloaca in Cryptobranchidae and Hynobiidae, whereas in the other taxa, the collecting ducts anastomose caudally and form a common collecting tube. This tube either communicates directly with the cloaca (Plethodontidae and some Salamandridae) or with the cloaca through the Wolffian duct (Ambystomatidae and some Salamandridae). These conditions arise in males because of a caudal migration of the collecting tubules during development (Rodgers and Risley, 1938). We term this the "complex condition."

In males, the epithelium lining the collecting duct tubules of the complex condition varies seasonally with reproductive activity in the Salamandridae (Aron, 1924; Adams, 1940; Miller and Robbins, 1954) and, perhaps, the Ambystomatidae (Norris, unpublished data, see Norris, 1987). During reproductive activity, secretory granules fill the apical regions of the collecting duct epithelium and the ducts nearly double in diameter (Aron, 1924; Adams, 1940; Miller and Robins, 1954). No function has been attributed to this increase in secretion synthesis (Norris, 1987), although the secretion has been previously referred to as "albuminoidiques" (Aron, 1924). However, considering their increased activity during the reproductive season, it appears as if the collecting ducts in salamanders (at least in the complex condition) have evolved a function as secondary sexual organs.

Besides the three investigations discussed above (Aron, 1924; Adams, 1940; Miller and Robins, 1954), no data exist on the potentially "sexual" collecting ducts in salamanders. Although the description is limited, the study by Sakai and Kawahara (1983) supplies an electron micrograph of the collecting duct epithelium. Here, we describe the histology, histochemistry, and ultrastructure of the nephron of *Ambystoma maculatum* in an effort to elucidate functional and morphological aspects of the collecting ducts in salamanders. We also review the literature on structures associated with the pelvic kidney and create synonymies for the historical terminology.

MATERIALS AND METHODS

Four male *Ambystoma maculatum* were utilized from the Saint Louis University Museum collection. Two were captured in a pond on March 18, 2010 in Crawford County, MO. Snout-vent lengths were 78 and 80 mm. These salamanders were injected with McDowell's-Trump fixative (Electron Microscopy Sciences, Hatfield, PA) and were submerged in the same fixative for 4 h. After initial fixation, the urogenital tracts were removed and submerged in a second solution of McDowell's-Trump fixative for 48 h. The left urogenital tract was then rinsed and dehydrated via increasing concentrations of ethanol (35, 70, 95, and 100%), differentiated with toluene, and then submerged in melted paraplast under vacuum for 12 h before embedding in paraffin. Transverse sections were sliced at 7 μm, affixed to slides with albumin, and stained with hematoxylin and eosin for general structural analysis, brilliant blue (BB) for protein concentration, or periodic acid-Schiff's (PAS) for neutral carbohydrates following the protocols of Kiernan (1990). Slides were viewed with a Leica DM4500 microscope (Leica Microsystems, Wetzlar, Germany) and digital micrographs were obtained via a Quicam 12-Bit Mono Fast 1394 Cooled digital camera (Qimaging Corporation, British Columbia, Canada). Images were subsequently uploaded into Adobe Creative Suite (Adobe Systems, San Jose, CA) for labeling. The other two salamanders were also collected in Crawford County, MO, in May; however, the year of collection was not reported. These salamanders were fixed in formalin, and their entire urogenital tracts were prepared as described above.

The right urogenital tracts of salamanders fixed in McDowell's-Trump fixative were rinsed in phosphate buffered saline (PBS; pH 7.4) and subsequently postfixed in 2% osmium tetroxide in PBS (pH 7.4). Tissues were then rinsed with PBS (pH 7.4), dehydrated via a graded series of ethanol (70, 85, 95, and 100%), submerged in a 1:1 mixture of ethanol and propylene oxide, followed by pure propylene oxide, and subsequently embedded in Epon (EmBed 812, Electron Microscopy Science, Hatfield, PA) for ultrathin sectioning with a Leica EM UC6 ultramicrotome (Leica Microsystems, Wetzlar, Germany). Sections of 75 nm were taken, placed on copper grids, and stained with uranyl acetate and lead citrate. Grids were then viewed with a JEOL JEM 100S TEM (JEOL USA, Peabody, MA) and photographed with a L3C CCD digital camera (Scientific Instruments and Applications, Duluth, GA). Images were subsequently uploaded into Adobe Creative Suite for labeling.

RESULTS
Gross Morphology and Nomenclature

Major structures of the adult male salamander kidney have been termed differently in previous investigations (Table 1). The pelvic kidneys rest dorsal to the pelvic bones, hence the terminology utilized here. On gross dissection of the venter, the kidneys can be observed; however, observation of the entire pelvic kidney apparatus is not possible as the collecting ducts travel laterally and then bend medially covering the remaining kidney structures (Fig. 1A,B). We utilize the terminology of collecting ducts due to the fact that no apparent collecting ducts are observed more proximally within the kidney. Any terminology that previously utilized "ureters" for these structures is inappropriate due to the lack of homology with the amniote ureters. Medial to the collecting ducts, the paired lightly pigmented Wolffian ducts travel ventral to the kidney. All of these structures lie retroperitoneal within the same pleuroperitoneum.

By pulling the collecting ducts laterally, the more proximal ducts of the kidney are exposed (Fig. 1B). The proximal kidney ducts are tightly coiled within a narrow medial portion of the kidney (Fig. 1B). These proximal ducts open into ~40, large, less-coiled, collecting ducts (Fig. 1B). The collecting ducts anastomose caudally and empty

TABLE 1. Ontology of the pelvic kidney structures from gross examination in salamanders

Proposed terminology	Historical terminology	Source(s)	Taxon(a)*	Definition
Pelvic kidney	Pars renalis	Blum, 1985	Textbook	Caudal portion of the kidney. The functional units of this portion of the kidney are the nephrons. The nephrons obtain filtrate from the blood stream through renal corpuscle and the coloemic cavity through the perotineal funnels.
	Lumbar kidney	Witchi, 1937; Rodgers and Risley, 1938; Adams, 1940	Ambystomatidae: Salamandridae	
	Definitive kidney	Francis, 1934; Baker and Taylor, 1964; Baker, 1965; Ratcliff, 1965; Willett, 1965; Strickland, 1966; Rosenquist and Baker, 1967	Ambystomatidae; Cryptobranchidae; Plethondontidae; Salamandridae; Sirenidae; Proteidae	
	Uriniferous kidney	Kent and Carr, 2001; Kardong, 2008	Textbook	
	Excretory kidney	Willett, 1965; Rosenquist and Baker, 1967	Sirenidae; Proteidae	
	Pelvic kidney	Spengel, 1876 (Beckenniere); Chase, 1923; Yamagiwa, 1924 (Beckenniere); Sakai and Kawahara, 1983	Hynobiidae; Proteidae; Salamandridae	
Collecting ducts	Collecting ducts	McCurdy, 1937; Witchi, 1937; Rogers and Risley, 1938; Adams, 1940; Strickland, 1966	Ambystomatidae; Salamandridae; Plethodontidae	Lateral tubules of the kidney in which the nephrons empty urine. In Proteidae and Sirenidae, these tubules empty into the Wolffian ducts multiple times along their lengths. In other salamander taxa, these tubules bend caudally and interact with the cloaca individually (e.g., Cryptobranchidae and Hynobiidae), anastomose and empty into the cloaca with the Wolffian duct (e.g., Ambystomatidae; some Salamandridae), or anastomose and empty into the cloaca (e.g., Plethodontidae; some Salamandridae). In at least Ambystomatidae, Plethodontidae, and Salamandridae, these tubules are secondary sexual organs.
	Renal-collecting ducts	Miller and Robbins, 1954	Salamandridae	
	Secondary urinary ducts	Blüm, 1985	Textbook	
	Urinary tubules	Baker and Taylor, 1964; Baker, 1965; Ratcliff, 1965; Willett, 1965; Strickland, 1966; Rosenquist and Baker, 1967	Ambystomatidae; Cryptobranchidae; Plethodontidae; Salamandridae; Sirenidae; Proteidae	
	Collecting tubules	Chase, 1923; Yamagiwa, 1924 (sammelröhrchen der beckenniere); Baker, 1945; Kent and Carr, 2001	Amphiumidae; Hynobiidae; Proteidae; Textbook	
	Ureters	Francis, 1934; Adams, 1940; Rosenquist and Baker, 1967; Sakai and Kawahara, 1983	Proteidae; Salamandridae	
	Urinary collecting tubules	Rosenquist and Baker, 1967	Proteidae	
	Urinary collecting ducts	Mintz, 1947	Ambystomatidae	
	Accessory urinary ducts	Kardong, 2008	Textbook	
Common collecting duct	Ureter	McCurdy, 1936; Witchi, 1936; Baker and Taylor, 1964; Strickland, 1966	Ambystomatidae; Salamandridae; Plethodontidae	The common duct resulting from the anastomosis of the collecting tubules.

*No terminology was given for structures associated with the pelvic kidney in Dicamptodontidae (DeMarco, 1952). There are currently no descriptions of the urogenital structures associated with the pelvic kidney in Rhyacotritonidae.

into the Wolffian ducts via common collecting ducts. These common collecting ducts have been previously termed ureters; however, this is inappropriate for the same reason as the misnomenclature of the collecting ducts.

Structural Organization of the Kidney

The nephron of male *Ambystoma maculatum* can be divided into six distinct histological regions leading sequentially from a renal corpuscle: (1) neck segment, (2) proximal tubule, (3) intermediate segment, (4) distal tubule, (5) collecting tubule, and (6) collecting ducts. The neck segment communicates with the coelomic cavity via a pleuroperitoneal funnel through the ventral kidney wall. The renal corpuscles are arranged in a longitudinal row and occupy an area between the midsagittal plane and medial aspect of the kidney. The neck region tubule branches from the lateral aspect of
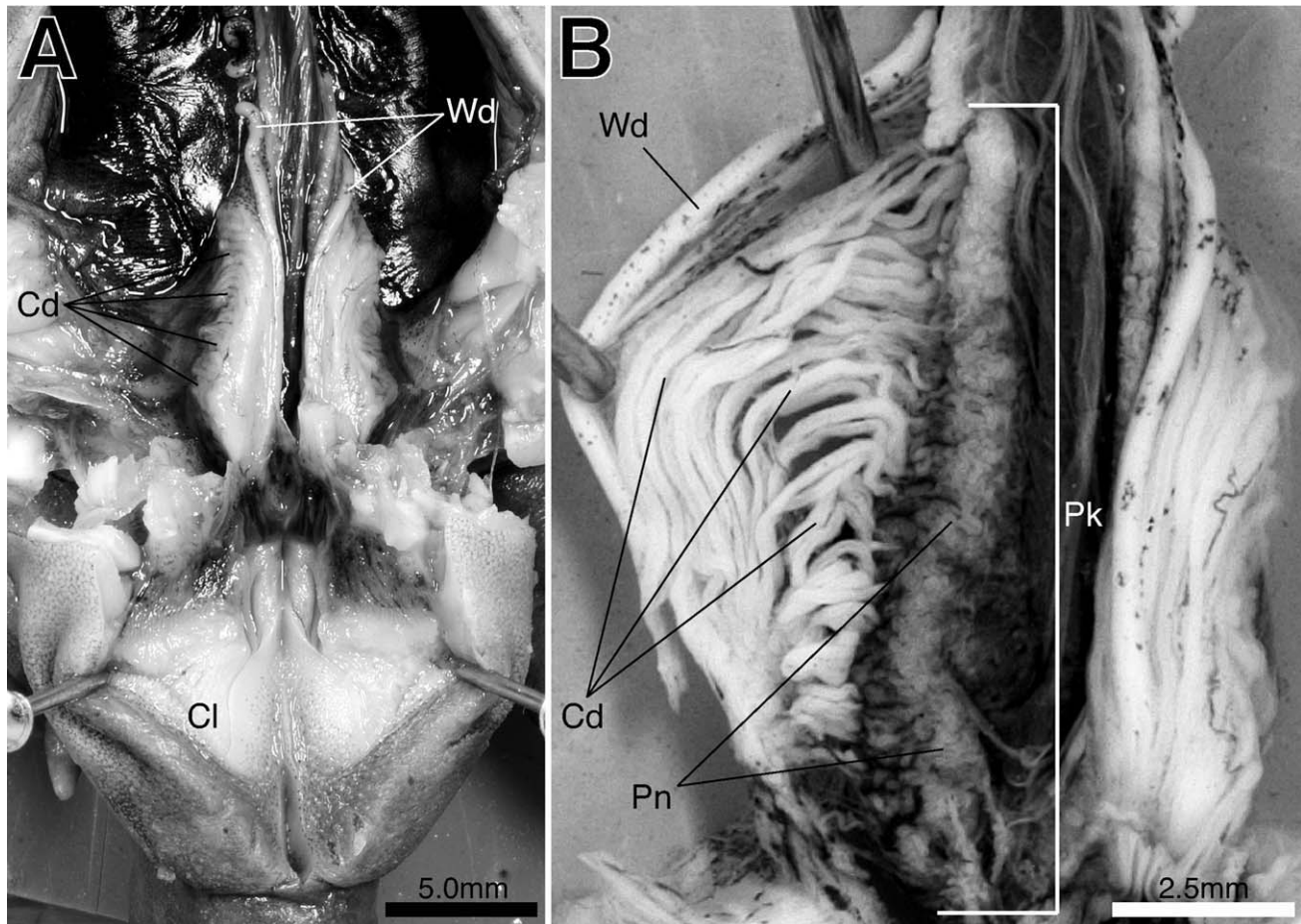
Fig. 1.   Gross anatomy of the caudal urogenital tract of male *Ambystoma maculatum*. **A:** Ventral overview of the pelvic kidney and cloaca highlighting the orientation of the intact kidney and ducts. **B:** High magnification of the pelvic kidney with the collecting ducts distended lateral/dorsal. Cd, collecting ducts; Cl, cloaca; Pk, pelvic kidney; Pn, proximal regions of the nephron; Wd, Wolffian duct.

the renal corpuscle and transitions into the proximal tubule at a more lateral aspect of the kidney. The proximal tubule possesses numerous convolutions in the lateral kidney before stretching medially and transitioning to the intermediate segment. The intermediate segment transitions to the distal tubule in the medial portion of the kidney. The distal tubule is less convoluted than the proximal and stretches laterally before transition to the collecting tubule and, subsequently, the collecting duct. The collecting ducts exit the kidney mass laterally but remain enclosed in a common serosa with the kidney. The collecting ducts have the greatest diameter and epithelial height ($\sim$220.3 and $\sim$40.5 μm, respectively) followed by the proximal tubules ($\sim$84.6 and $\sim$23.2 μm), distal tubules ($\sim$50.7 and $\sim$15.3 μm), and the ciliated regions ($\sim$41.5 and $\sim$12.3 μm).

Histology and Ultrastructure

Renal Corpuscle. The renal corpuscle 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 a visceral epithelium, which is in close association with the capillaries of the glomerulus (Fig. 2A). Parietal epithelial cells are squamous, have narrow intercellular canaliculi, and are adhered by desmosomes along the entire canaliculi length (Fig. 2B). The nuclei of the parietal epithelium are highly heterochromatic, and small round mitochondria and electron lucent vesicles of varying sizes are dispersed throughout the cytoplasm (Fig. 2B). Parietal cells rest against a basal lamina and share a lamina propria with the distal convoluted tubules (Fig. 2B). The epithelial layers of the renal corpuscle stain basophilic with no reaction to BB or PAS.

The visceral epithelium of the Bowman's capsule is squamous, modified into podocytes, and abuts the basal lamina of the glomerular capillaries (Fig. 2C). Adjacent podocytes are adhered by desmosomes, and desmosomes also join nonadjacent podocytes that are brought into close association
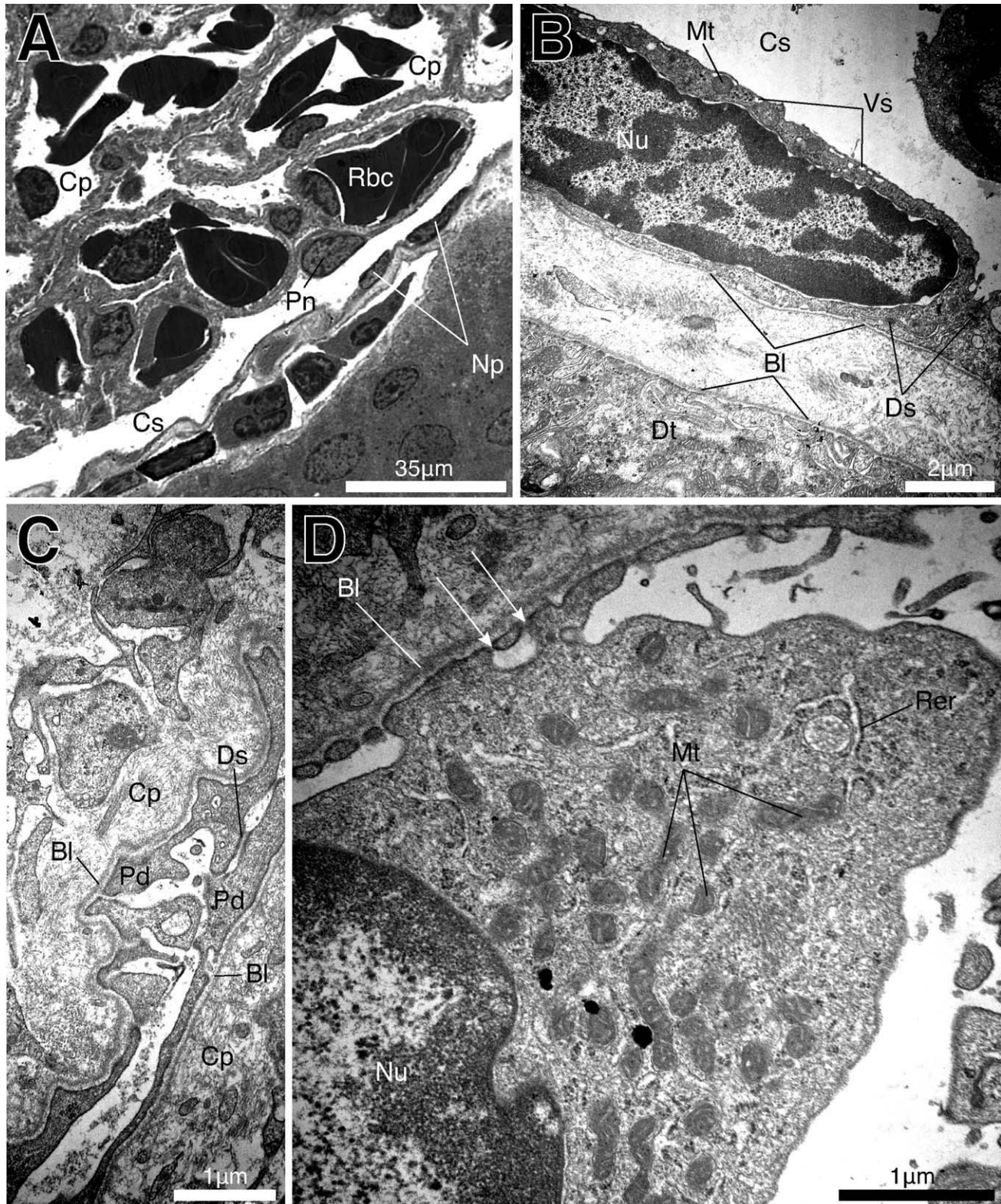
Fig. 2.   Fine structure of the renal corpuscle of male *Ambystoma maculatum*. **A:** Low magnification of a renal corpuscle (toluidine blue). **B:** High magnification of the parietal epithelium of a renal corpuscle (uranyl acetate and lead citrate). **C:** High magnification of the visceral epithelium of a renal corpuscle (uranyl acetate and lead citrate). **D:** High magnification of the cytoplasmic contents of a podocyte (white arrows indicate filtration slits; uranyl acetate and lead citrate). Bl, basal lamina; Cp, capillary; Cs, capsular space; Dt, distal tubule; Ds, desmosomes; Go, Golgi complex; Mt, mitochondria; Np, nuclei of parietal epithelium; Nu, nuclei; Pd, podocyte; Pn, podocyte nuclei; Rbc, red blood cell; Rer, rough endoplasmic reticulum; Vs, vesicles.
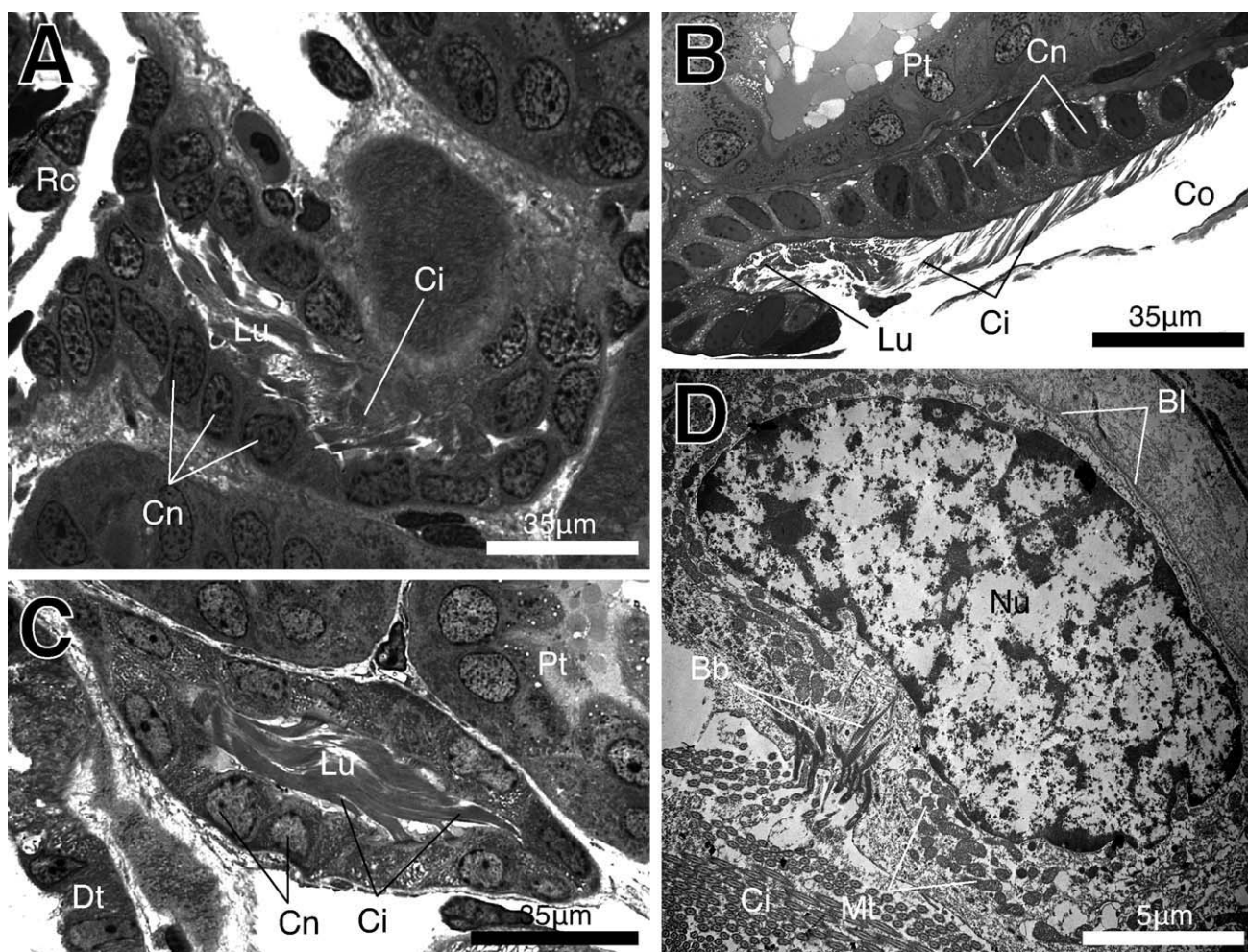
Fig. 3. Structure of the neck region (**A**), pleuroperitoneal funnel (**B**), and intermediate segment (**C**), of male *Ambystoma macula-tum* (toluidine blue). **D:** High magnification of a ciliated cell from the intermediate segment (uranyl acetate and lead citrate). Bb, basal bodies; Bl, basal lamina; Ci, cilia; Cn, ciliated cell nuclei; Co, coelom; Dt, distal tubule; Lu, lumen; Mt, mitochondria; Pt, proximal tubule.

by the convolutions of the glomerular capillaries (Fig. 2C). Nuclei are heterochromatic (Fig. 2D). Small and elongated mitochondria fill the perinuclear space of the podocytes and profiles of rough endoplasmic reticulum, and lucent vesicles of varying sizes are also common throughout the cytoplasm (Fig. 2D). A mildly electron-dense material fills the lumina of larger vesicles (Fig. 2D). The basal portion of the podocytes is modified into footlike projection and creates fenestrations along the walls of the glomerular capillaries (Fig. 2D).

Neck Region, Intermediate Segment, and Pleuroperitoneal Funnel. The parietal epithelium of the renal corpuscle is continuous with the epithelial lining of the nephron (Fig. 3A). The lumen of the nephron is continuous with the capsular space (Fig. 3A). The first region of the nephron distal to the renal corpuscle is the ciliated neck region. The epithelium of this region is simple cuboidal with centrally located nuclei (Fig. 3A).

All of the cells of the neck region are ciliated and only two other regions of the nephron possess an epithelium completely lined by cilia: (1) the pleuroperitoneal funnel (Fig. 3B) and (2) the intermediate segment (Fig. 3C) between the proximal and distal tubules. The epithelium of all of these regions is basophilic with no reaction to PAS and only an apical reaction of the ciliated cells with BB. The cytology of all of these regions is identical; however, from 1 μm thick sections it appears that the nuclei of the pleuroperitoneal funnel epithelial cells stain slightly darker with toluidine blue (Fig. 3B), and the cilia of the intermediate segment are noticeably longer than the other respective regions (Fig. 3C). Thus, the cytological description below encompasses all of the completely ciliated nephron regions.

Ciliated cells of the aforementioned regions are filled apically with small, slightly elongated mitochondria, and lucent vacuoles are often inter-
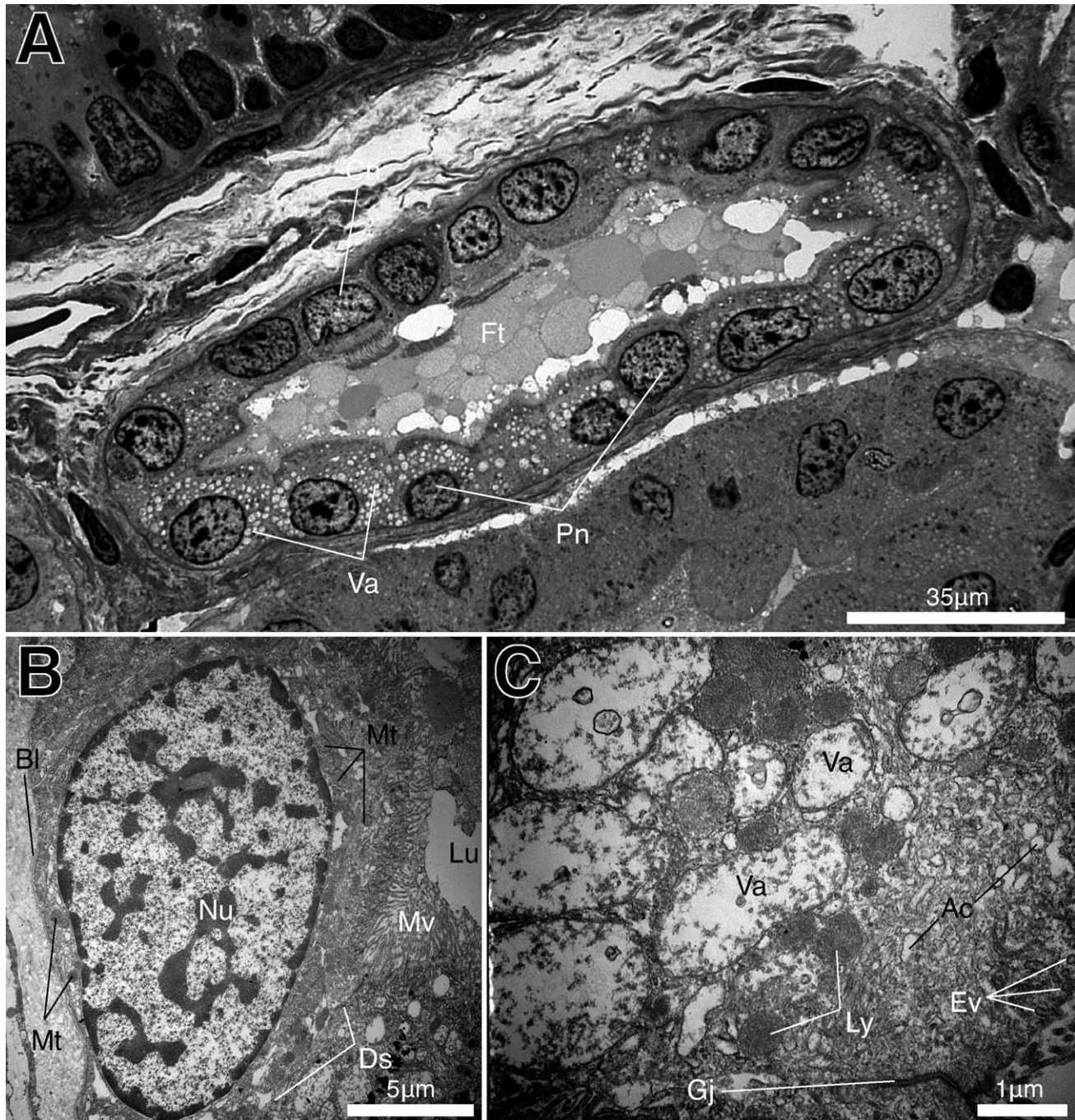
Fig. 4. Fine structure of the early proximal tubule of male *Ambystoma maculatum*. **A:** Low magnification of the epithelium of the early proximal tubule (toluidine blue). **B:** High magnification of a principle cell in the early proximal tubule (uranyl acetate and lead citrate). **C:** High magnification of the apical cytoplasm from a cell in the early proximal tubule (uranyl acetate and lead citrate). Ac, apical canaliculi; Bl, basal lamina; Cn, ciliated cell nuclei; Ds, desmosome; Ev, endocytic vesicles; Ft, filtrate; Gj, gap junction; Lu, lumen; Ly, lysosome; Mt, mitochondria; Mv, microvilli; Nu, nuclei; Pn, principle cell nuclei; Va, vacuoles.

spersed with the mitochondria (Fig. 3D). Elongated cilia anchor to basal bodies in the central portion of the apical cell surface (Fig. 3D). The peripheral region of the apical cell surface is smooth (Fig. 3D). Intercellular canaliculi are narrow and nontortuous. Apical tight junctions and distal des-

mosomes adhere to adjacent ciliated cells. The basal plasma membrane is unmodified and contours to the basal lamina (Fig. 3D).

Proximal Tubule. The proximal tubule can be divided cytologically into two distint regions: (1) a proximal vacuolated region (Fig. 4A) and (2) a dis-
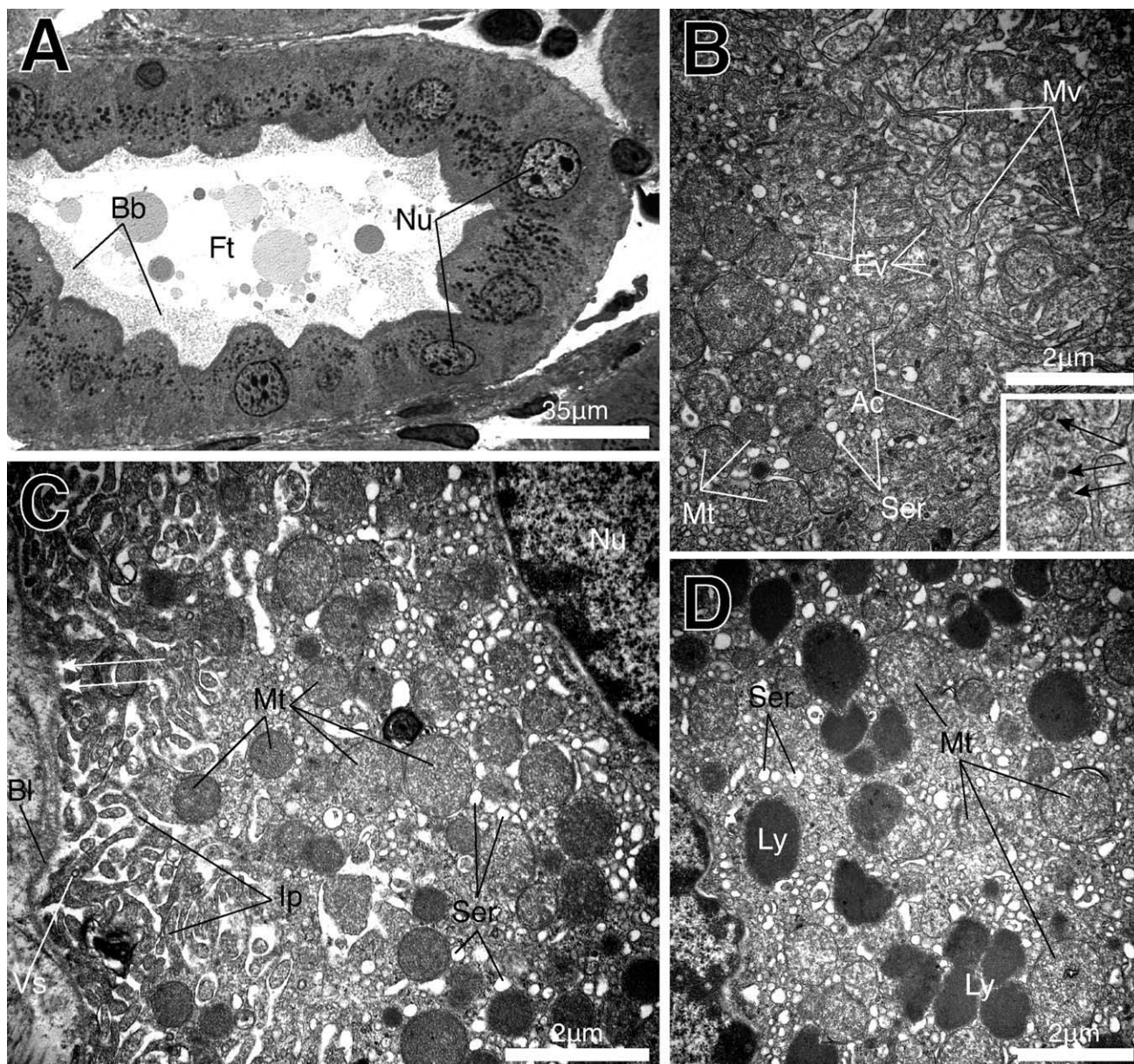
Fig. 5. Fine structure of the late proximal tubule of male *Ambystoma maculatum*. **A:** Low magnification of the late proximal tubule (toluidine blue). **B:** High magnification of the apical cytoplasm of a proximal tubule cell and apical endocytic vesicles (inset; uranyl acetate and lead citrate). **C:** High magnification of the basal portion of a proximal tubule cell (white arrows indicate fenestrations between the foot processes of the basal plasma membrane; uranyl acetate and lead citrate). **D**. High magnification of the supranuclear region of a proximal tubule cell (uranyl acetate and lead citrate). Ac, apical canaliculi; Bb, brush border; Ev, endocytic vesicles; Ft, filtrate; Ip, interditigating processes; Ly, lysosomes; Mt, mitochondria; Mv, microvilli; Nu, nuclei; Ser, smooth endoplasmic reticulum; Vs, vesicles.

tal region studded with electron-dense bodies. The vacuolated region is easily identified by shorter apical microvilli (Fig. 4B) than the more distal region, numerous vacuoles that fill the apices of the epithelial cells (Fig. 4C), a basal cell membrane that lacks complex folding (Fig. 4B), few dense lysosomes (Fig. 4C), scant profiles of smooth endoplasmic reticulum, and a few ciliated cells with identical morphology to those of the neck

(Fig. 4A). The epithelium is also shorter and, therefore, the nuclei appear centrally positioned (Fig. 4B). The stereotypical vacuoles of this region are lucent with scattered diffuse material within (Fig. 4C). The epithelium of the entire proximal tubule is eosinophilic. Filtrate from the glomerular capillaries fills the more proximal portions of this region (Fig. 5A), which is eosinophilic and stains positive with BB. The filtrate is made up of large

globular substances of varying electron densities and smaller granular substances (Figs. 4A and 5A).

The distal region of the proximal tubule possesses a simple columnar epithelium with basal heterochromatic nuclei (Fig. 5A). The central aspect of the apices of the epithelial cells is domed, and long microvilli cover the apices of every epithelial cell (Fig. 5A,B). Between the apical microvilli, intracellular canaliculi invaginate into the epithelial cells, and endocytic vesicles are observed below the surface of the apical plasma membrane (Fig. 5B and inset). Smooth endoplasmic reticulum is abundant throughout the epithelial cells (Fig. 5B–D), whereas large circular mitochondria aggregate beneath the apical and basal plasma membrane (Fig. 5B,C). Electron-dense lysosomes are restricted to the supranuclear space of the proximal tubular epithelial cells (Fig. 5D), and these inclusions stain positive with PAS. The lateral plasma membrane and intercellular canaliculi are distinct with little digitation. Desmosomes adhere to the adjacent epithelial cells along the entire length of the intercellular canaliculi, whereas tight junctions are present at its apical extremity with a gap junction located more basally. Numerous invaginations form small foot-like projections at the basal region of the plasma membrane creating a fenestrated membrane region atop the basal lamina (Fig. 5C). The basal foot processes are highly interdigitating. Small lucent vesicles are often present at the distal tips of the foot-like projections (Fig. 5C).

Distal tubule and collecting Tubule. The lumen of the distal tubule is filled with filtrate; however, the filtrate is more homogeneous than that observed in the more proximal tubules (Fig. 6A). The epithelium of the distal tubule is simple cuboidal with centrally positioned nuclei (Fig. 6A). Epithelial cell cytoplasm is basophilic with a weak reaction to BB. Nuclei are either heterochromatic or mostly euchromatic with dense aggregations of chromatin dispersed around the nuclear periphery (Fig. 6A,B). Mitochondria fill the cytoplasm of these cells and partially occlude the observation of other cytoplasmic contents (Fig. 6B). Mitochondria are oriented perpendicular to the apical plasma membrane and are highly elongated. Invaginations of the basal plasma membrane form very deep intracellular canaliculi basally (Fig. 6C). This causes the basal foot processes of the epithelial cells to take on an almost filamentous appearance, with the distal ends of the filaments abutting the basal lamina (Fig. 6C). A highly dense material occupies the cytoplasm of the distal extremity of the foot processes (Fig. 6C). The basal foot processes do not interdigitate to the extent of the proximal tubule.

Microvilli are common on the apical surface of the cells but not to the extent of the proximal tubules. Adjacent epithelial cells of the distal tubule are adhered by an apical tight junction with one slightly more basal desmosome (Fig. 6D). The intercellular canaliculi basal to the junctional complexes are highly convoluted from lateral microvillus projections with interdigitating lamellae from every epithelial cell (Fig. 6D). Dispersed between mitochondria, profiles of smooth and rough endoplasmic reticulum are present. Small lipid droplets are often observed basal to the nuclei. Little cellular variation exists along the length of the distal tubules. Mitochondria in the more proximal regions of the distal tubule are larger, less numerous, and are less organized perpendicular to the basal lamina. The foot processes are also larger resulting in a decrease in the basal membrane surface area.

The collecting tubule has often been described as a portion of the distal tubule (see Discussion); however, many differences are observed. In terms of cytoplasmic contents, collecting tubule cells are identical to those of the distal tubule. Nuclei tend to be less round and more irregularly shaped (Fig. 6E), and less-pronounced labyrinths exist between the basal plasma membrane and basal lamina and laterally between adjacent epithelial cells. Mitochondria are also less numerous. Odd cells that stain deep purple with toluidine blue are also found intercalated between the majority light cells (Fig. 6E). The only prominent feature of these dark cells is numerous small mitochondria and a dark euchromatic nucleus (Fig. 6E).

Collecting Ducts. The collecting ducts are lined by tall columnar principle cells with basal heterochromatic nuclei (Fig. 7A). Ciliated cells are scattered in between the apices of adjacent columnar principle cells (Fig. 7A). The lumen of the collecting duct is difficult to observe in 1-μm sections stained with toluidine blue due to its dark staining contents that are identical in staining intensity to the numerous secretory granules found in the collecting duct epithelium (Fig. 7A).

Rough endoplasmic reticulum is abundant in the perinuclear region, and its cisternae are filled with a diffuse material in the principle cells of the collecting ducts (Fig. 7B,C). A large supranuclear Golgi complex is stereotypical (Fig. 7B). Invaginations of the basal plasma membrane are scarce; however, interdigitating microvillus projections line the lateral aspects of the principle cells (Fig. 7D). The principle cells rest on the basal lamina of multiple layers of fibroblasts and collagen fibers (Fig. 7C).

The large prominent electron-dense granules of the principle collecting duct cells are formed from fusion of condensing vacuoles with transport vesicles from the Golgi complex (Fig. 7D). Mature granules dominate the apices of the collecting duct epithelial cells, and this granulated region makes up about 4/5 of every cell (Fig. 7A). Mature granules are homogeneously electron dense (Fig. 8A,B),
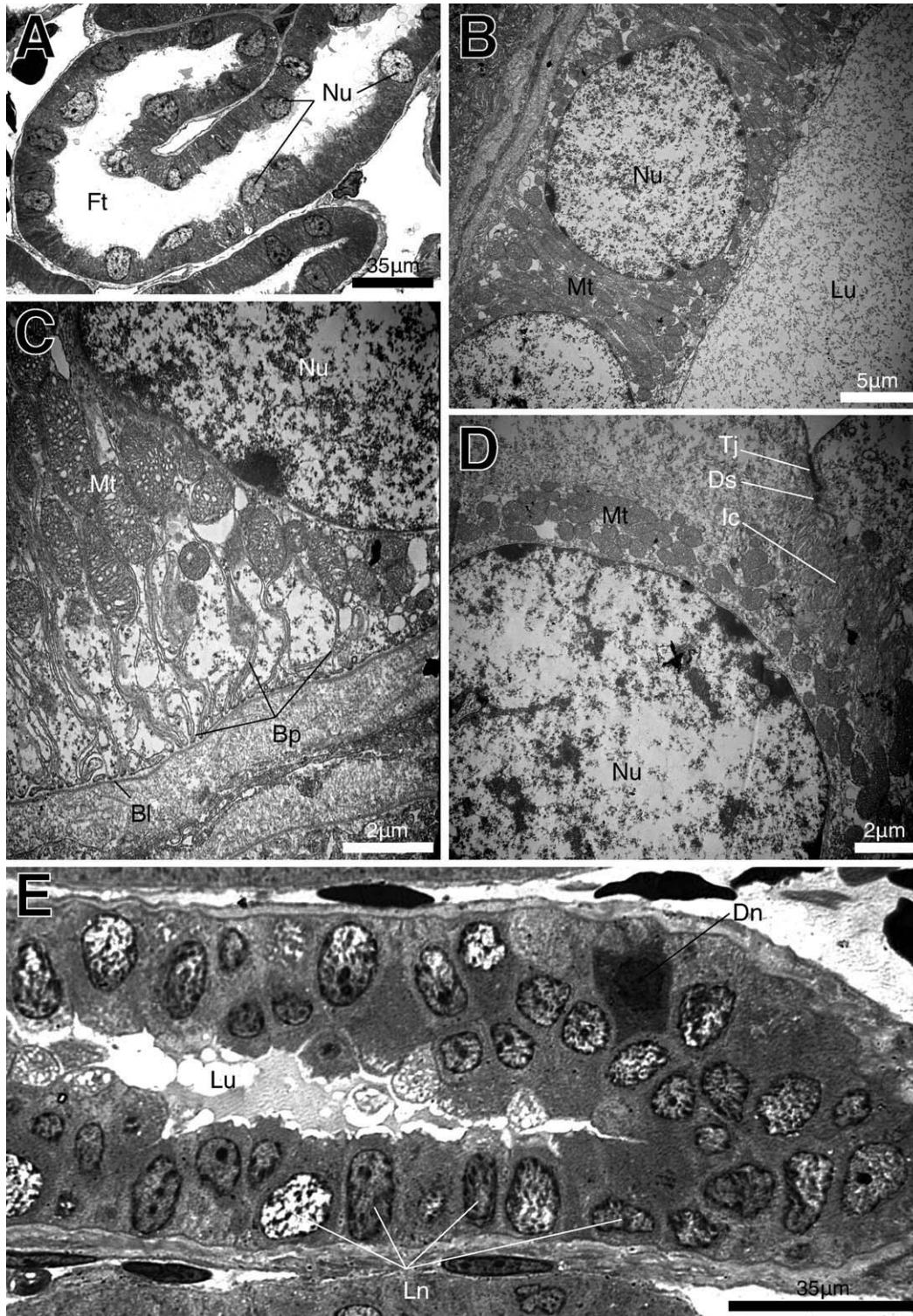
Fig. 6.   Fine structure of the distal tubule and collecting tubule of male *Ambystoma maculatum*. **A:** Low magnification of the distal tubule (toluidine blue). **B:** High magnification of the loan cell type in the distal tubule (uranyl acetate and lead citrate). **C:** High magnification of the basal portion of a distal tubule cell (uranyl acetate and lead citrate). **D:** High magnification of the supranuclear region of a distal tubule cell (uranyl acetate and lead citrate). **E:** Low magnification of the collecting tubule (toluidine blue). Bl, basal lamina; Bp, basal processes; Dn, dark-cell nuclei; Ds, desmosome; Ft, filtrate; Ic, intercellular canaliculi; Ln, light-cell nuclei; Lu, lumen; Mt, mitochondria; Nu, nuclei; Tj, tight junction.
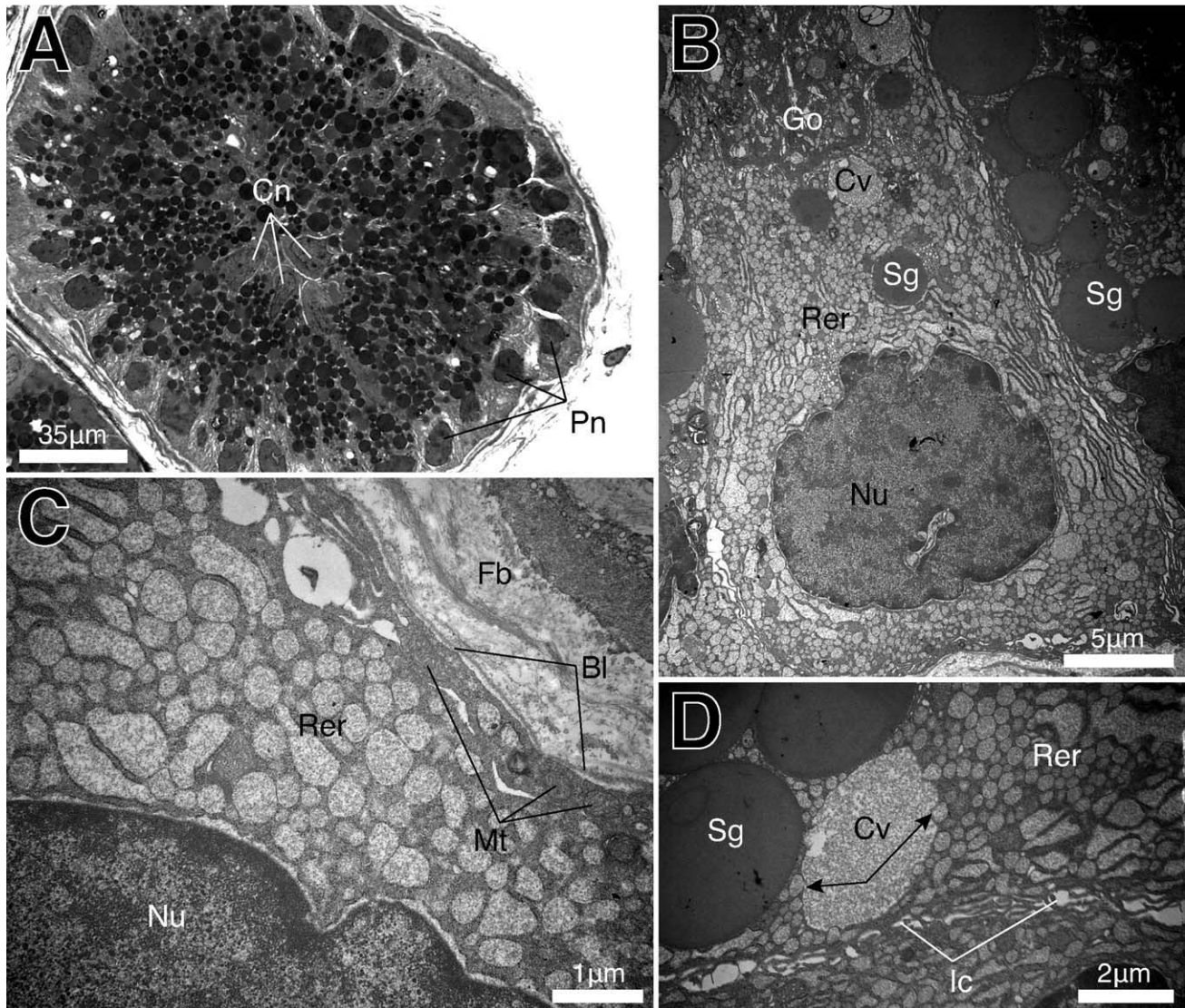
Fig. 7.   Fine structure of the sexual collecting ducts of male *Ambystoma maculatum*. **A:** Low magnification of a sexual collecting duct (toluidine blue). **B:** High magnification of the perinuclear region of a principle cell of the sexual collecting duct epithelium (uranyl acetate and lead citrate). **C:** High magnification of the basal portion of a sexual collecting duct principle cell (uranyl acetate and lead citrate). **D:** High magnification of the lateral aspects of two adjacent principle cells of the sexual collecting duct epithelium (uranyl acetate and lead citrate). Bl, basal lamina; Cn, ciliated cell nuclei; Cv, condensing vacuoles; Fb, fibroblast; Ic, intercellular canaliculi; Mt, mitochondria; Nu, nuclei; Pn, principle cell nuclei; Rer, rough endoplasmic reticulum; Sg, secretory granule.

are eosinophilic, are stain positive with PAS, and have a weak reaction to BB. In some principle cells, small to large cellular inclusions fill spaces between secretory granules (Fig. 8A,B). These inclusions are mildly electron dense, nonuniform, and typically have scattered regions of highly electron-dense material internally (Fig. 8A,B). In extreme cases, entire principle cells are filled with an inclusion. The cellular inclusions and mature secretory granules are released into the lumen via merocrine secretion (Fig. 8B); however, immature and mature secretory granules are also found in the collecting duct lumen (Fig. 8B) indicating the

possibility of cellular rupture or an apocrine mode of secretion.

The cytology of the apical ciliated cells is identical to the ciliated cells of the previously discussed ciliary regions (Fig. 8C) with few exceptions. Unlike the ciliated nephron regions, the ciliated cells in the collecting ducts do not abut a basal lamina but instead rest on convex corners of adjacent principle cells. Microvillus projections from the basal portions of the ciliated cells interdigitate with the lateral membranes of the principle cells and are adhered apically with the principle cells by an apical tight junction and one slightly more
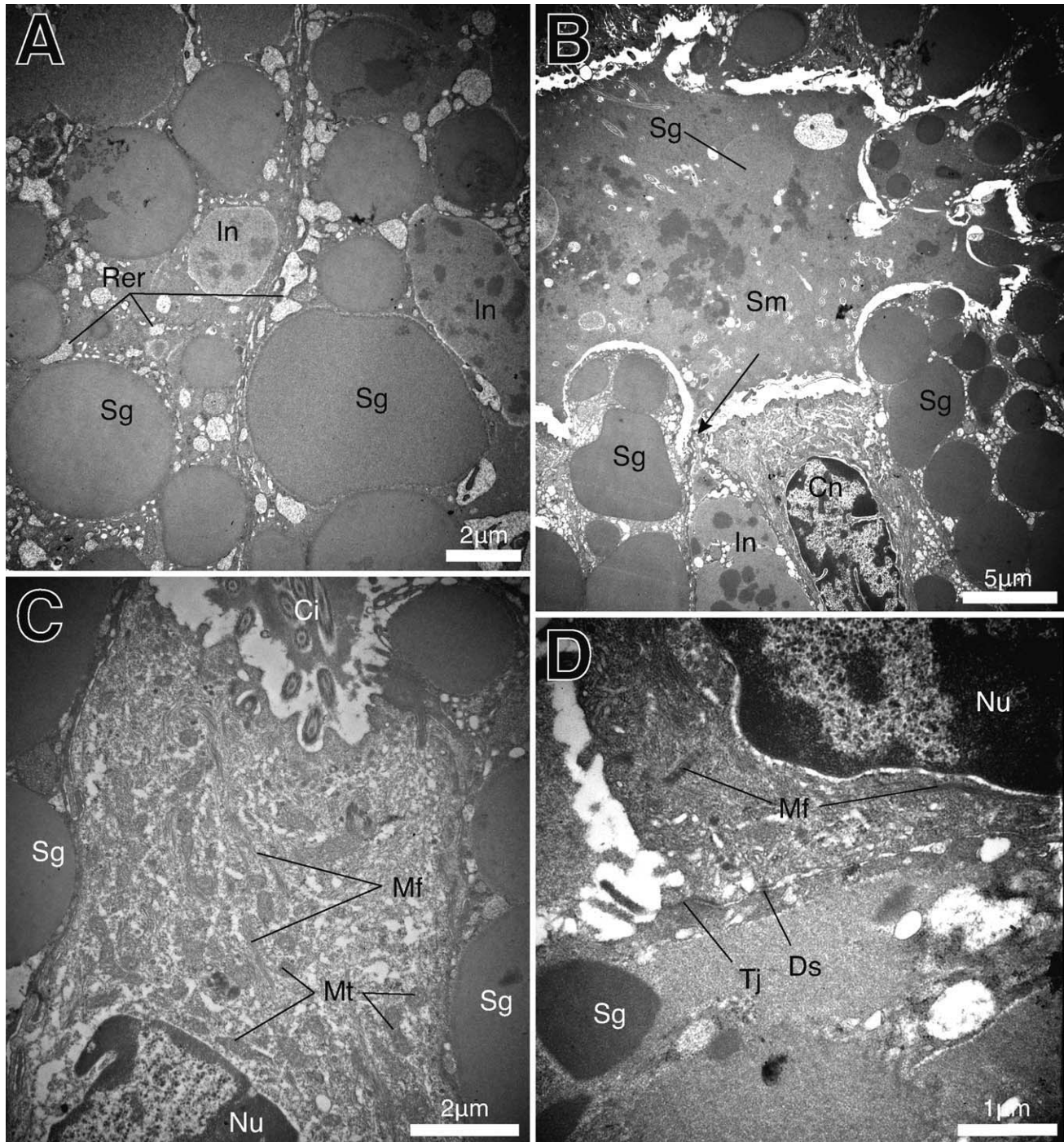
Fig. 8. Fine structure of the sexual collecting ducts of male *Ambystoma maculatum*. **A:** High magnification of the supranuclear region of a sexual collecting duct principle cell (uranyl acetate and lead citrate). **B:** High magnification of the apical portion of a sexual collecting duct principle cell (black arrow indicates a merocrine type secretion in process; uranyl acetate and lead citrate). **C:** High magnification of the apical region of a ciliated cell in the sexual collecting duct (uranyl acetate and lead citrate). **D:** High magnification of the lateral aspects of adjacent principle and ciliated cells of the sexual collecting duct (uranyl acetate and lead citrate). Ci, cilia; Cn, ciliated cell nuclei; In, cellular inclusions; Mf, microfilaments; Nu, nuclei; Rer, rough endoplasmic reticulum; Ds, desmosome; Sg, secretory granule; Sm, secretory material; Tj, tight junction.

basal desmosome (Fig. 8D). Cilia also cover the entire surface of cells of the collecting ducts (Fig. 8C).

Wolffian Duct. Like the collecting ducts, the Wolffian ducts are lined by a layer of principle cells that are simple tall columnar (Fig. 9A). The
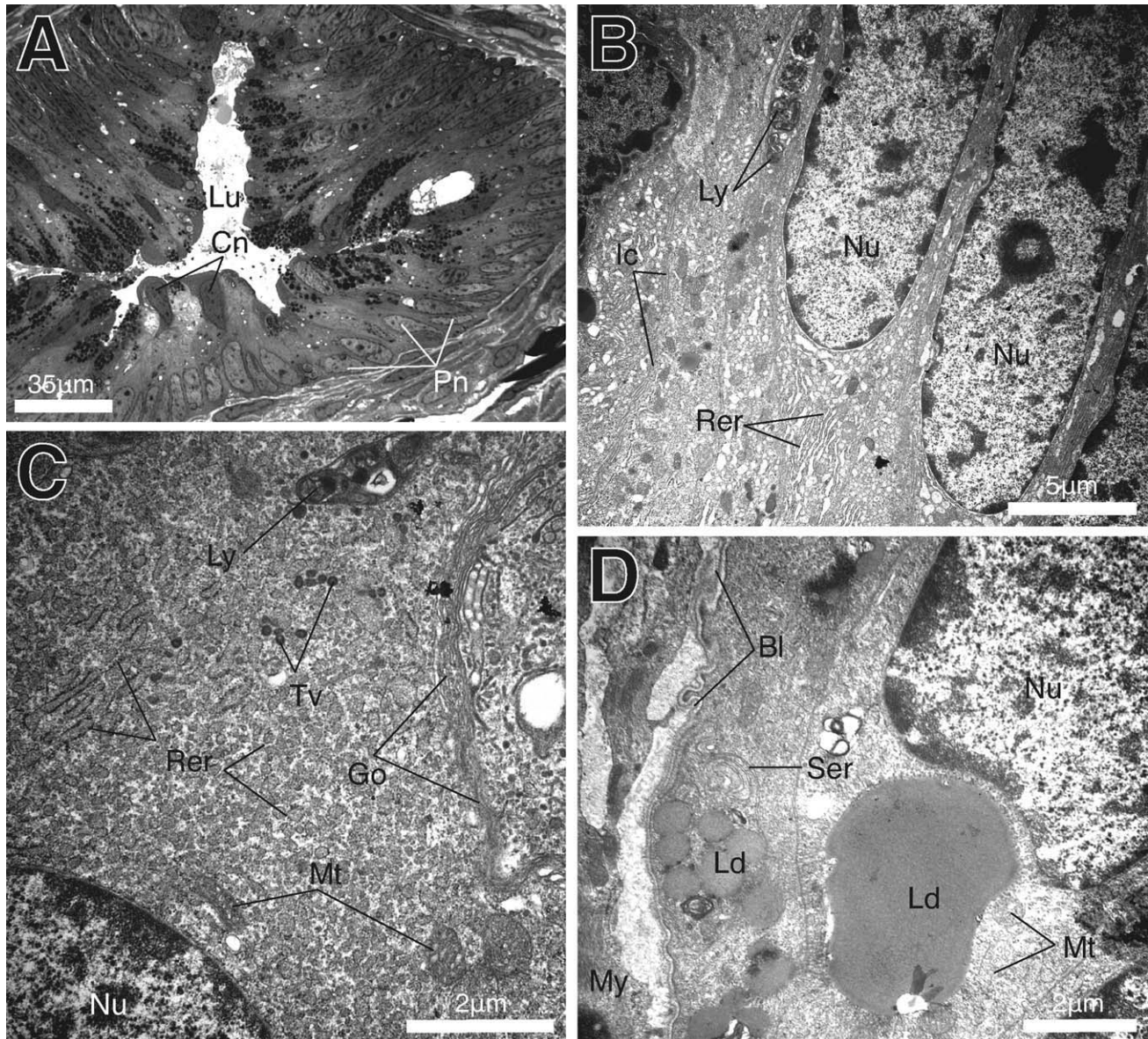
Fig. 9. Fine structure of the Wolffian duct of male *Ambystoma maculatum*. **A:** Low magnification of a Wolffian duct (toluidine blue). **B:** High magnification of the supranuclear region of principle cells from the Wolffian duct (uranyl acetate and lead citrate). **C:** Higher magnification of the supranuclear region of a principle cell of a Wolffian duct (uranyl acetate and lead citrate). **D:** High magnification of the basal portion of a Wolffian duct principle cell (uranyl acetate and lead citrate). Bl, basal lamina; Cn, ciliated cell nuclei; Go, Golgi complex; Ic, intercellular canaliculi; Ld, lipid droplets; Lu, lumen; Ly, lysosomes; Mt, mitochondria; Nu, nuclei; Pn, principle cell nuclei; Rer, rough endoplasmic reticulum; Ser, smooth endoplasmic reticulum; Tv, transport vesicles.

epithelial cells have a narrow width and ciliated cells rest in between the apices of adjacent principle cells. Nuclei of both cell types are located in a basal position and are heterochromatic with those of the ciliated cells being slightly darker than those of the principle cells in 1 μm thick toluidine blue stained tissues (Fig. 9A). The cytoplasm of the Wolffian duct epithelium is basophilic.

The principle cells possess abundant profiles of rough endoplasmic reticulum that are most dense supranuclear (Fig. 9B). Electron-dense transport vesicles from supranuclear rough endoplasmic reticulum are abundant in the cytoplasm basal to the trans region of a medially oriented Golgi complex (Fig. 9C). Lysosomes are also common in the principle cell cytoplasm and aggregate along the intercellular canaliculi (Fig. 9B,C). Intercellular canaliculi are narrow, and few interdigitating microvillus projections are present between adjacent cells (Figs. 9B and 10A). Profiles of smooth endoplasmic reticulum are observed infranuclear and are often in association with lipid droplets of
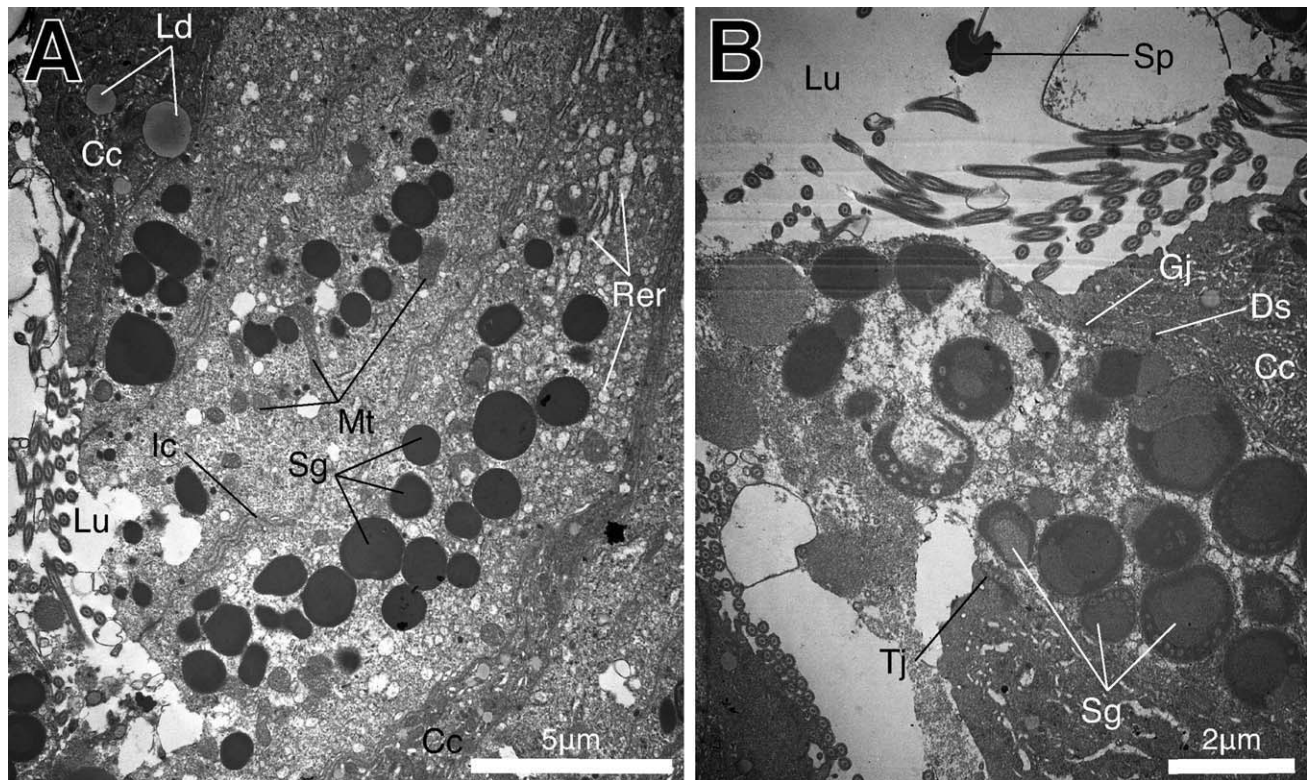
Fig. 10. Fine structure of the Wolffian duct of male *Ambystoma maculatum*. **A:** High magnification of the apical potion of a Wolffian duct principle cell (uranyl acetate and lead citrate). **B:** Higher magnification of the apparent secretory process of a Wolffian duct epithelial cell (uranyl acetate and lead citrate). Cc, ciliated cell; Ds, desmosome; Ic, intercellular canaliculi; Gj, gap junction; Ld, lipid droplets; Lu, lumen; Mt, mitochondria; Rer, rough endoplasmic reticulum; Sg, secretory granules; Tj, tight junction.

varying diameters (Fig. 9D). The principle cells rest against a basal lamina with no villus modifications of the basal cell membrane (Fig. 9D).

Like the principle cells of the collecting ducts, the principle cells of the Wolffian ducts are filled with electron-dense secretory granules that stain positive with PAS but weakly with BB; however, these granules are much smaller in diameter (Fig. 10A). Dark elongated mitochondria are also abundant in the apical region of the principle cells (Fig. 10A). The secretory process of the Wolffian duct principle cells is odd and can most accurately be termed atypical apocrine. The apical cell membrane appears to bulge and rupture, and the apical granules degranulate and become vesicular (Fig. 10B). Ciliated cells of the Wolffian duct are identical in structure to those found in the collecting ducts, although basal lipid droplets are often found in the Wolffian duct ciliated cells.

DISCUSSION
Nephron

The nephron of male *Ambystoma maculatum* was similar in structure to that of other amphibians previously described (for review, see Møbjerg et al. 2004). Thus, we restrict our discussion to sal-amander nephron morphology, as we provide little data that add to the functional or morphological evolution of the vertebrate nephron. However, we refer readers to Møbjerg et al. (2004) and Maunsbach and Boulpaep (1984), who provide comparative qualitative and quantitative analyses with nonurodelan taxa. Most studies on amphibian nephron morphology have described a renal corpuscle, neck region, proximal tubule, intermediate segment, distal tubule, collecting tubules, and collecting ducts (Møbjerg et al., 1998; Møbjerg et al., 2004).

In comparison with the proximal tubule of *Ambystoma tigrinum, Amphiuma means* (termed proximal segment), *Necturus maculosus*, and *Triturus pyrrhogaster*, the distal portion of the proximal tubule of *Ambystoma maculatum* is practically identical. The apical border is covered entirely by microvilli, and lysosomes (reportedly with acid phosphatase activity) fill the cytoplasm in all taxa in at least one section of the proximal tubule (Clothier et al., 1978; Sakai and Kawahara, 1983; Maunsbach and Boulpaep, 1984). The lysosomes of *Ambystoma tigrinum* appear slightly larger and aggregate lateral to the nucleus (Maunsbach and Boulpaep, 1984), whereas, in *Ambystoma maculatum, Amphiuma means*, and *T. pyrrhogaster* lyso-

somes are noticeably smaller, more numerous, and only fill the supranuclear space. In *Ambystoma maculatum* and *Ambystoma tigrinum*, the basal extracellular labyrinth is much more pronounced than the lateral intercellular space (Maunsbach and Boulpaep, 1984). Maunsbach and Boulpaep (1984) hypothesized that the morphology of the basal membrane lends itself to the rapid exchange of water and solute between the basal labyrinth and the peritubular space. In *Amphiuma means*, *N. maculosus*, and *T. pyrrhogaster*, the lateral and basal membrane labyrinths are much less pronounced (Clothier et al., 1978; Sakai and Kawahara, 1983; Maunsbach and Boulpaep, 1984). Because of the aquatic nature of these taxa, this finding highlights decreased requirement for water resorption in the proximal tubule.

Neither Sakai and Kawahara (1983) nor Maunsbach and Boulpaep (1984) described a proximal portion of the proximal tubule with large vacuoles in *Ambystoma tigrinum*, *N. maculosus*, or *T. pyrrhogaster*. Whether this was due to its absence, oversight, or the notion that this vacuolated region represented a transitional region between the neck and the proximal tubule is unknown. Clothier et al. (1978) noted three regions of the proximal tubules (region 2 was discussed above) in *Amphiuma means*. Region 1 (most proximal) possessed few cellular inclusions, whereas region 3 (most distal) possessed abundant lucent vacuoles similar to those observed in the most proximal region of the proximal tubule in *Ambystoma maculatum*. From the Figure 11 of Clothier et al. (1978), it is clear that this region differs from that of the most proximal region in *Ambystoma maculatum*, as the vacuoles occupy the entire cytoplasm of each epithelial cell. The large vacuoles most likely highlight the storage capability in regions of the proximal tubule in *Ambystoma maculatum* and *Amphiuma means*().

Heterogeneity in function and morphology has previously been described in the distal tubule of *Ambystoma tigrinum* (Hinton et al., 1982). Two regions of the distal segment were described for this taxon: (1) a diluting segment and (2) a junctional segment (Hinton et al., 1982). Morphology of the diluting segment was identical to the description provided for the distal tubule of *Ambystoma maculatum* above, *Amphiuma means* [termed distal segment (Clothier et al., 1978) or late distal segment (Stanton et al., 1984)], and *T. pyrrhogaster* (Sakai and Kawahara, 1983). The junctional segment possessed pale cells with less-organized mitochondria and reduced interdigitations between adjacent pale cells (Hinton et al., 1982) like that of the collecting tubule and junctional segment described for *Ambystoma maculatum* and *Amphiuma means*, respectively. The proximal portion actively transported chloride and had a positive luminal voltage, whereas the junctional segment actively transported sodium and had a

negative luminal voltage (Hinton et al., 1982). Sakai and Kawahara (1983) did not note any variation along the length of the distal tubule in *T. pyrrhogaster*; however, they described a segment that they termed the collecting duct, which is similar in morphology to the junctional segment and collecting tubule (Clothier et al., 1982; Hinton et al., 1982). Sakai and Kawahara (1983) utilized the nomenclature of ureters for what we have termed the collecting ducts.

Stanton et al. (1984) described two distinct regions in the distal tubule/diluting segment (not including the collecting tubule) in *Amphiuma means*: (1) early/diluting segment and (2) late segment. No other study divided the region that we term the distal tubule into two discrete sections; however, the variation that Stanton et al. (1984) observed was similar to the variation we observed in the distal tubule between the proximal and distal portions. We did not feel that this variation warranted delineation of a new region, as we perceived this region as a transitional phase from the intermediate segment to the distal tubule. Stanton et al. (1984) also believed that this region was discrete, came into contact with the renal corpuscle, and may be a rudimentary junxtaglomerular apparatus. A previous study aimed to describe a juxtaglomerular apparatus in *Ambystoma mexicanum* failed to distinguish definitive specialization of the distal tubule, although, it is unclear if much attention, or any, was assigned to the distal tubule (Hanner and Ryan, 1980). The variation in *Amphiuma means* in comparison with other salamanders may represent a discrete difference in nephron structure between groups of salamanders.

Variation along the length of the distal tubules and collecting duct system has led to rather inconsistent terminology adopted for the distal regions of the nephron, as various amphibian taxa possess different arrays of cellular types (Møbjerg et al., 1998, 2004). However, it is now clear that at least two distinct regions exist between the intermediate segment and collecting ducts in salamanders. We feel that the historic distinctness of these two regions warrants definitive terminology for the regions. Thus, we prefer the terminology of a distal tubule and collecting tubule to the terminology of a distal tubule with diluting and junctional segments. The terminology of Sakai and Kawahara (1983) is inconsistent with any other study as the distal tubule/diluting segment and collecting tubule/junctional segment were termed the distal tubule and collecting duct, respectively. Ontology of salamander nephron nomenclature established from ultrastructural investigations is provided in Table 2.

Collecting Ducts

The collecting ducts of salamanders with the complex condition have been implicated as second-

TABLE 2. *Regions of the nephron in salamanders delineated with ultrastructural investigation*

Ambystoma maculatum	*Ambystoma tigrinum*	*Ambystoma tigrinum*[1]	*Amphiuma means*	*Amphiuma means*	*Triturus pyrrhogaster*
This study	Hinton et al., 1982	Maunsbach and Baulpaep, 1984	Clothier et al, 1978	Stanton et al., 1984	Sakai and Kawahara, 1983
Neck segment	NA	NA	Narrow segment	NA	Neck segment
Proximal tubule	NA	Proximal tubule[2]	Proximal segment	NA	Proximal tubule
Vacuoloated region			Region 1		
Lysosomic region			Region 2		
			Region 3		
Intermediate segment	NA	NA	Connecting segment	NA	Intermediate segment
NO	NO	NO	NO	Early distal tubule/diluting segment	NO
Distal tubule	Diluting segment	NA	Distal segment	Late distal segment	Distal tubule
Collecting tubule	Junctional segment	NA	Junctional segment	Collecting tubule	Collecting duct
Collecting duct	NA	NA	Collecting duct	NA	Ureter

"NA" refers to not applicable. For example, these regions were not investigated in the respective studies. "NO" refers to not observed in the respective studies.
[1]Maunsbach and Baulpaep (1984) also investigated the kidney of *Necturus maculosus* for comparison with *Ambystoma tigrinum*; however, the majority of the ultrastructural description was from *A. tigrinum*. The only variation noted was the occasional cell without apical microvilli and the decreased volume of the basal extracellular labyrinth in the proximal tubule of *N. maculosus*. No nomenclatural differences were presented.
See Discussion for review of the morphology of each region.
[2]No regional variation described.

ary sexual structures in the salamandrids *Ichthyosaura alpestris* (Aron, 1924), *Notophthalmus viridescens* (Adams, 1940), and *Taricha torosa* (Miller and Robbins, 1954). In all of these species, the collecting ducts were found to either increase secretory activity or increase in size in concordance with their reproductive seasons. Norris (1987) also cited similar activity in the collecting ducts of Ambystomatidae from unpublished data. Although a seasonal collection was not obtained due to the difficulty of collecting ambystomatid salamanders outside of their reproductive season, we are comfortable confirming the unpublished data of Norris (1987), as it appears that the collecting ducts of ambystomatid salamanders also produce a sexual secretion. At least three of the seven families of salamanders, including *Plethodon albagula* (unpublished data) that have the complex pelvic kidney condition, have collecting ducts with secondary sexual activity.

No detailed description of the sexual collecting ducts exists. In his account, Aron (1924) described the epithelium of the collecting ducts as nonciliated, simple, and tall columnar that contained numerous eosinophilic granules. Adams (1940) described the collecting ducts of *Notophthalmus viridescens* as similar to the Wolffian ducts with a similar seasonal cycle. Sakai and Kawahara (1983) provided an ultramicrograph of the collecting duct epithelium from another salamandrid, *T. pyrrhogaster*; however, no detail on the microstructure was given besides that the epithelium possessed numerous electron-dense granules. Some variation clearly exists between the collecting ducts of salamandrids, ambystomatids, and plethodontids. Although two cell types (ciliated and secretory) make up the epithelium of *Ambystoma maculatum*, only one cell type (secretory) exists in plethodontids and salamandrids (Aron, 1924; Sakai and Kawahara, 1983). The granules that make up the majority of the collecting duct epithelial cells in ambystomatids and salamandrids are eosinophilic (Aron, 1924; Adams, 1940). Beyond these two comparisons, little is known on the diversity found within collecting duct structure and secretion in salamanders; however, we found that the epithelium is distinct from that of any other nephron region or the Wolffian ducts. The collecting ducts function solely in secretion, which is evidenced by the lack of expansion of the apical, lateral, and basal membranes. They also lack lipids as observed in the Wolffian duct epithelium and possess distinctly larger secretory granules than those observed in the Wolffian duct epithelium.

Nephrons with secondary sexual function in males have only been described in three taxa of vertebrates. In gasterosteids (stickleback fishes), the highly secretory region of the ventral kidney ducts produces a protein called "spiggin" that is the major constituent of the foam nests of stickle-

back fishes (Courrier, 1922; Craig-Bennet, 1931; Jakobsson et al., 1999). In squamates (lizards + snakes; Fox, 1977), and possibly all lepidosaurians (Tuatara + Squamata; Gabe and Saint-Girons, 1964), the highly secretory sexual segment of the kidney is formed in either the distal nephron tubules or collecting ducts (Fox, 1977). The secretions function in copulatory plug formation in some taxa (Devine, 1975; Ross and Crews, 1977; Nilson and Andrén, 1982) and have also been proposed to activate sperm (Bishop, 1959; Cuellar, 1966), may produce pheromones (Volsøe, 1944), and/or may contribute to the seminal fluid (Prasad and Reddy, 1972). In salamanders, the sexual unit of the kidney is restricted to the collecting ducts, although the function of the secretions produced by the collecting ducts is unknown. Kidneys with secondary sexual function most likely evolved independently in vertebrates, and the restriction of sexual function to the distal portion of the nephrons in all of the above taxa highlights the flexibility in function of the distal nephron tubules in vertebrates. In contrast, the similarities in sexual segment structure and chemistry highlight evolutionary restrictions in the functional morphology of the collecting ducts.

Although the function of the secretions from the sexual collecting ducts in salamanders is unknown, we provide some insight into possible utilities. The secretion could produce a component of the spermatophore that is used to transfer sperm to the female cloaca after courtship. However, Sever and Houck (1985) described all the components of the spermatophore arising from the glands of the male cloaca in *Desmognathus ochropheus* (Plethodontidae); yet, they did not investigate the sexual collecting ducts. Considering the collecting ducts never come into contact with the Wolffian ducts (where sperm are stored) until immediately cranial to where the urogenital papillae insert into the urodaeum, it is possible that the secretion serves to activate sperm. Sperone et al. (2009) reported that sperm removed from the Wolffian ducts are immotile, indicating possible support for the latter hypothesis. Histological sections through the cranial extremities of the cloaca of male *Plethodon albagula* during spermatophore formation revealed the mixing of sperm and secretions from the collecting ducts (unpublished data). We are confident that the secretion is not albumen as indicated by Aron (1924) because of the highly eosinophilic staining of the epithelial granules. Obviously much work is required to elucidate the functional and evolutionary implications of kidneys with secondary sexual function in salamanders, and we refrain from offering further conjectures until more data are gathered on this subject.

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