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Morphology of the Kidney in the West African Caecilian, *Geotrypetes seraphini* (Amphibia, Gymnophiona, Caeciliidae)

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ABSTRACT This study deals with the morphology and ultrastructure of the mesonephros in adult caecilians of the species *Geotrypetes seraphini*. Based on serial sections in paraffin and araldite, nephrons are reconstructed and the cellular characteristics of different nephron segments described. The long and slender mesonephric kidneys of *G. seraphini* are broadest caudally and taper toward the front, where the organs are divided into smaller segmental divisions. Two nephron types can be distinguished on the basis of their connections to the coelom and their position within the nephric tissue: *ventral nephrons* connect to the coelom via a ciliated peritoneal funnel, whereas *medial nephrons* lack this connection. Both nephron types are composed of a filtration unit, the Malpighian corpuscle, and a renal tubule, which can be divided into six morphologically distinct segments: neck segment, proximal tubule, intermediate segment, early distal tubule, late distal tubule, and collecting tubule. Collecting tubules merge and form a branch system that opens into collecting ducts. Collecting ducts empty into the Wolffian duct. Proximal tubules of nephrons in the frontal divisions are morphologically different from the proximal tubules of more caudal kidney regions. Distal tubule subdivision is only clearly recognizable at the electron microscopic level. The length of each nephron segment is calculated from a ventral nephron with a total length of ~3.8 mm, and the course of the segments within the nephric tissue is reported. The number of nephrons was estimated at 1,700 units in each kidney. The segmentation and ultrastructure of the mesonephric nephrons in *G. seraphini* are discussed in relation to nephron descriptions from other caecilians and we further discuss the evolutionary origin of the amphibian nephron. J. Morphol. 262:583–607, 2004.

KEY WORDS: Amphibia; *Geotrypetes seraphini*; Gymnophiona; mesonephros; morphology; nephron; ultrastructure

During vertebrate ontogenesis three different kidney forms appear, i.e., the pronephros, mesonephros, and metanephros. Each of these kidneys is paired and is located on the roof of the body cavity, ventrally covered by the cavity's epithelium. Although the kidney forms differ in overall organization and complexity, they all have the nephron as the basic structural and functional unit (Goodrich, 1958; Saxén, 1987).

Within the amphibian life cycle two kidney forms are present and functional (for recent summaries, see Vize et al., 1997; Møbjerg et al., 2000; Vize, 2003). The amphibian kidney systems, pronephroi of larvae and mesonephroi of both larvae and adults, are important organs for excretion and osmoregulation. The amphibian pronephros resembles to a high degree the metanephridial system of invertebrates, whereas the mesonephros is more complicated and forms a link between these systems and the more specialized metanephric kidneys of amniotes. The amphibian kidney therefore provides the opportunity to study very different levels of kidney complexity and amphibian kidney systems have been essential to the understanding of vertebrate kidney development, as well as nephron function (see, e.g., reviews by Guggino et al., 1988; Dietl and Stanton, 1993; Vize et al., 1997; Brändli, 1999; Drummond and Majumdar, 2003; Schultheiss et al., 2003; Vize et al., 2003).

The kidney of caecilian amphibians is often viewed as being almost representative of the ancestral vertebrate kidney, and studies of caecilian kidneys are therefore often used in discussions on vertebrate kidney evolution. However, detailed recent studies of caecilian kidneys are sparse.

The gross morphology of the mesonephros in the caecilians, as studied so far, is very similar to that of other amphibians and it has a mesonephric segmental form (see discussions by Wake, 1970; Sakai and Kawahara 1983; Sakai et al., 1986). Wake (1970)

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summarizes the gross kidney morphology and gives dimensional data on the filtration unit and renal tubules of different caecilian species. Little is known of the morphology and ultrastructure of the nephron in different caecilian genera. Welsch and Storch (1973) gave an ultrastructural account of the nephron in *Ichthyophis kohtaoensis* without focusing on overall nephron structure. Carvalho and Junqueira (1999) present light microscopical data on the histology of the kidney and urinary bladder of *Siphonops annulatus*. A thorough structural study has been conducted on the kidney of the Neotropical caecilian *Typhlonectes compressicauda* (see Sakai et al., 1986, 1988a, b). However, this species is a highly derived, secondarily aquatic form with a nephron that is unlikely to be representative of the ancestral caecilian nephron (Wilkinson and Nussbaum, 1999). In understanding the caecilian nephron and its relationships to that of other amphibians we therefore need to appreciate diversity within caecilians.

Here we present the morphology and ultrastructure of the kidney in adults of the West African caecilian *Geotrypetes seraphini*, a terrestrial caeciliid caecilian. *Geotrypetes* and *Typhlonectes* are members of a clade of "higher" caecilians that includes the Caeciliidae, Typhlonectidae, and Scolecomorphidae, but are otherwise not closely related (Wilkinson, 1997; Wilkinson et al., 2003). This is the first thorough study of the mesonephric nephron in a terrestrial caecilian. The analysis is conducted with the aid of light and electron microscopy on serial kidney sections. On this basis, nephrons are reconstructed and the cellular characteristics of different nephron segments are described. Emphasis is placed on the nephron's connection to the coelom and on differences in nephron segmentation along the caudofrontal axis of the kidney. We compare nephron structure in *G. seraphini* with the structure of nephrons in other amphibians and caecilians in particular, and we discuss the evolutionary origin of the amphibian nephron.

MATERIALS AND METHODS

Three specimens of *Geotrypetes seraphini* (A. Duméril, 1859), said to be from Cameroon, were obtained commercially. The specimens, referred to as Specimens 1–3, are deposited in the collections of the Zoological Department, The Natural History Museum, London, UK (reg. nos. BMNH 2000.226-2000.228).

Only female caecilians were used in the present study, as they lack the connections between kidney and gonadial systems—the urogenital connections—which in males may imply modifications of nephrons used in sperm transport. The cytology of the nephron and the urogenital connections in male *Geotrypetes seraphini* will be reported in a later study (Møbjerg, Jespersen, Wilkinson, in prep.). Prior to fixation, Specimen 1, with a body length of 16.0 cm and a kidney length of 8 cm, and Specimen 2, with a body length of 19.5 cm and a kidney length of 9 cm, were kept under terrestrial conditions in moist soil with access to tap water and are referred to as the terrestrial specimens. In these specimens the lumen of the kidney tubules was often collapsed. Amphibians kept in a freshwater environment produce copious and very dilute urine. Out of water, however, glomerular filtration may cease altogether and this would lead to a collapse of the renal tubule. Specimen 3, with a body length of 21.0 cm and a kidney length of 10 cm, was therefore kept overnight in tap water before preservation for the histological examination and is referred to as the aquatic specimen.

Animals were anesthetized by immersion in tap water containing MS222 and were subsequently decapitated. After opening the body cavity, the internal organs were perfused through the ventricle or aorta with an aldehyde fixative composed of 1.2% glutaraldehyde, 1.0% paraformaldehyde, 0.05 mol/l sodium cacodylate buffer, 0.05 mol/l sucrose, pH 7.4. To ensure that the kidney tissue was fully embedded in fixative, a second perfusion through the vena cava was performed in Specimens 1 and 3. Whole animals were subsequently left in fixative overnight, ensuring that the kidneys were fully exposed to the solution. Specimens were rinsed in a 0.05 mol/l sodium cacodylate buffer, pH 7.4 containing 0.05 mol/l sucrose and transferred to the buffer for storage. Small pieces of kidney were removed and either dehydrated through ethanol and tetrahydrofuran and embedded in paraffin or postfixed in 1% OsO_4 in 0.1 mol/l sodium cacodylate buffer for 1 h, dehydrated in ethanol and propylene oxide, and embedded in araldite. Paraffin sections, 8 μm thick, were stained with hematoxylin-eosin; 2-μm araldite sections were stained with toluidine blue. Semithin sections were examined using a light microscope. Ultrathin araldite sections were contrasted with uranyl acetate and lead citrate and examined in Jeol 100SX and Jeol 1010 transmission electron microscopes (TEM).

Semithin serial sections of the araldite- and the paraffin-embedded kidney tissue were used for reconstructions of nephrons. The reconstructions were performed using a light microscope to trace the course of the nephron segments in successive sections. The 3D reconstruction presented in Figure 5 is based on serial araldite sections from the frontal region of the left kidney in Specimen 1 (Figs. 1, 2). The nephron was reconstructed by projecting the contours of the different segments from consecutive sections onto paper and thereby creating a 3D illustration. The artistic representation of the reconstruction was later prepared by a professional illustrator. The araldite section series contained both semithin and ultrathin sections, enabling us to determine the precise cytology by TEM of the nephron segments identified under the light microscope. Figures 3 and 17 were made in CorelDraw 10 (Corel, Canada). Summary data are given as mean ± SEM. In measurements on Malpighian corpuscle size, *n* equals the number of measured corpuscles.

RESULTS

General Kidney Morphology (Figs. 1–4)

The mesonephric kidneys of *Geotrypetes seraphini* are long, slender organs located retroperitoneally on either side of the aorta and vena cava (Figs. 1, 2). The kidney is broadest caudally and tapers toward the front. In the frontal region it is characterized by segmental constrictions, which divide the organ into smaller divisions, each composed of a cluster of nephrons (Figs. 1, 2). Caudally, the kidney almost reaches the anterior part of the cloaca. Ovaries are situated in the mesovarium along the medial side of the middle part of the kidney (Fig. 2). Fat bodies are located alongside the ovaries in the same mesentary and extend all the way to the caudal part of the kidney (Fig. 2). The renal portal vein and the Wolffian duct (ureter) run along the lateral side of the kidney. The Wolffian duct extends from the first renal segment along the entire length of the kidney and finally leaves the lateral edges and opens into the dorsal wall of the cloaca just anterior to the

Fig. 1. A kidney dissected from *Geotrypetes seraphini* (Specimen 1). LM. **A:** Dorsal view of the dissected kidney. The mesonephric kidneys are long, slender organs located on either side of the aorta and vena cava. Fat bodies located alongside the kidney have been partially removed (compare with Fig. 2). The kidneys are broadest caudally and taper toward the front. **B:** The frontal region is characterized by segmental constrictions, which divide the organ into smaller divisions. **C:** Magnification of caudal region of the kidneys. Note the ramifications of the laterally situated renal portal veins spreading dorsally. Scale bar = 5 mm (**A**).

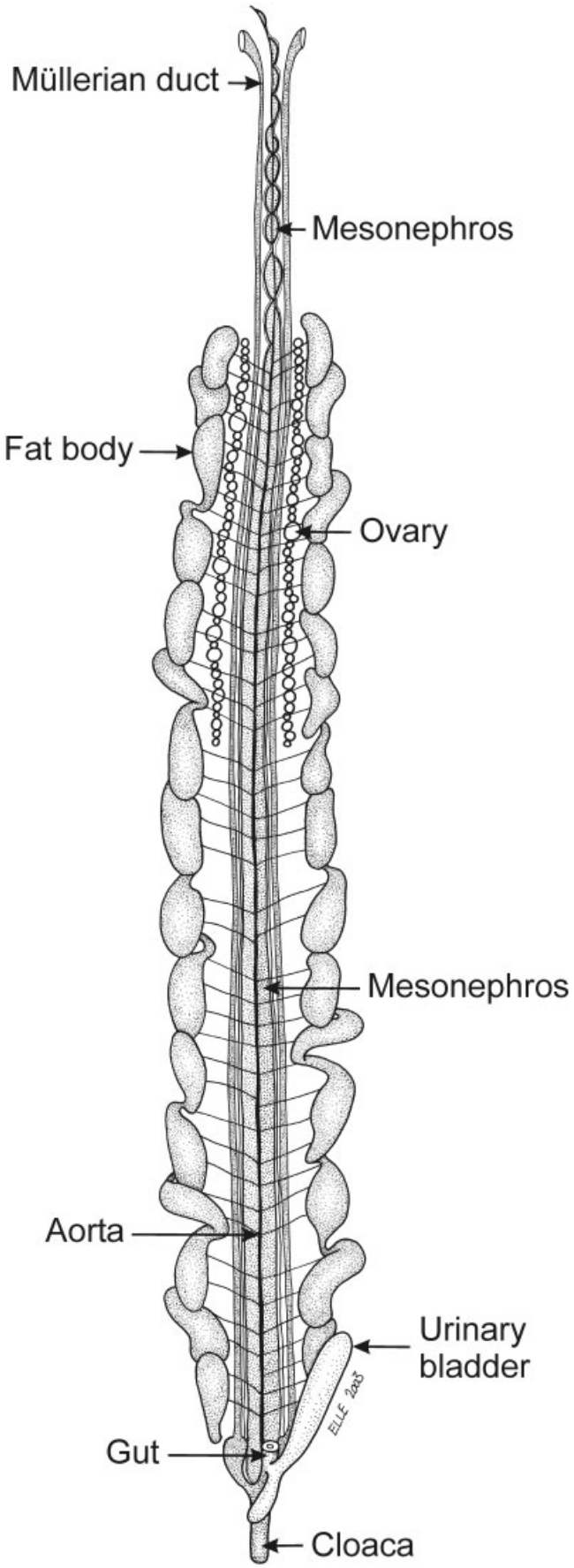
opening of the ventrally located urinary bladder (Fig. 2).

Reconstructions of the structural and functional unit of the kidney, the nephron, were made from serial transverse and longitudinal sections. The nephron is composed of a filtration unit, the Malpighian corpuscle, and a renal tubule, which can be divided into six morphologically distinct segments: neck segment, proximal tubule, intermediate segment, early distal tubule, late distal tubule, and collecting tubule (Fig. 3). Collecting tubules merge and form a branch system that opens into dorsolaterally located collecting ducts, which empty into the Wolffian duct.

In the *Geotrypetes* kidney we found two types of nephrons—in *ventral nephrons* the Malpighian corpuscles are situated along the ventral surface of the kidney and in *medial nephrons* the Malpighian corpuscles are located more dorsally along the medial axes of the kidney. From the ventral surface of the kidney, peritoneal funnels connect to the neck segment of ventral nephrons through a ciliated tubule (Fig. 3). The ciliated tubules are very narrow at their distal ends, in some instances only composed of two cells with a height of less than 2 μm and no visible lumen. The funnels often appear in pairs, each connecting to one or two ventral nephrons. Medial nephrons lack this connection to the coelom (Fig. 3), and in the midfrontal to caudal part of the kidney these nephrons have larger Malpighian corpuscles and clearer distal tubule segmentation (see below). In the frontal region of the kidney large ventral Malpighian corpuscles are found. In medial nephrons a ciliated tubule also leaves the neck segment, but the tubule ends blindly or connects to an internal blind funnel often linking 3–4 Malpighian corpuscles (Fig. 3). The total number of nephrons was estimated by counting the number of filtration units in araldite and paraffin sections of different regions of the left kidney from Specimen 1. In this specimen the number of nephrons was estimated at 1,700 units in each kidney.

When viewed in transverse sections the kidney can be divided into a *ventromedial zone* and a *dorsolateral zone* (Fig. 4). The ventromedial zone is characterized by the presence of Malpighian corpuscles, distal tubules, and peritoneal funnels with their ciliated tubules along with the neck and intermediate segments of ventral nephrons. The dorsolateral zone is dominated by the presence of proximal tubules and tubules of the collecting duct system. From longitudinal sections it is clear that within these two zones there are differences in the distribution of the different segments along the caudofrontal axes. In the ventromedial zone the Malpighian corpuscles of both ventral and medial nephrons group together in clusters associated with the neck segments, the peritoneal funnels and ciliated tubules of ventral nephrons. This is also true for the tubules of the collecting duct system in the dorsomedial zone. The collecting tubules of a cluster of nephrons open into the branch system at approximately the same point, leaving the space between such branching points to be filled mostly by proximal tubules. Malpighian corpuscle clusters start with one or two ventral corpuscles situated caudal to a cluster of medial corpuscles. The number of the latter depends on the kidney region with more nephrons found in cross sections of the broader caudal part of the kidney.

Blood enters the kidney from two points, the renal portal veins and the renal arteries. Blood flows



through the kidney in a dorsolateral–ventromedial direction. Ramifications of the laterally situated renal portal vein spread dorsally and create the peritubular capillary system surrounding the nephrons. Renal arteries, which supply the afferent arterioles, branch from the aorta, and enter the kidney from the medial margin. Each Malpighian corpuscle receives one afferent arteriole and a single efferent arteriole leaves the filtration unit. The afferent arterioles of both ventral and medial nephrons enter the Malpighian corpuscles medially. In medial nephrons the efferent arterioles exit at approximately the same point, while efferent arterioles of ventral nephrons often leave the corpuscles more dorsally. In ventral nephrons the efferent arterioles often have a smaller diameter than the afferent arteriole, while there are no differences in medial nephron arteriolar size. Efferent arterioles of both nephron types run along the dorsal surface of the Malpighian corpuscle in a lateral direction and empty into peritubular capillaries. Blood leaves the kidney through efferent veins entering the vena cava from the medial margin (Fig. 4).

Nephron Structure (Figs. 2, 5, 6)

Unless otherwise noted, the following description and measurements are based on a reconstruction of a ventral nephron from the midfrontal part of the left kidney just anterior to the region of the ovaries from Specimen 1 (Fig. 5). The kidney of this specimen was 8 cm long and had a caudal height of 0.5 mm and width of 1 mm, with decreasing width toward the front (see Fig. 2). In this region of the kidney approximately one-third of the nephrons are ventral nephrons. The total length of the reconstructed nephron was measured as ~3.8 mm. In this terrestrial specimen the lumina of many of the renal tubules were collapsed and this is reflected in a low outer diameter, especially of proximal tubules and tubules of the collecting duct system.

The Malpighian corpuscle is situated on the ventral border of the kidney in the ventromedial zone and measures 95 × 60 μm, when viewed from mediolateral and dorsoventral margins, respectively. The afferent arteriole enters the corpuscle medially, while the smaller efferent arteriole leaves the corpuscle more dorsally (Fig. 5A). This corpuscle is relatively small when compared to the corpuscles of the surrounding medial nephrons. The maximal size of the corpuscle in medial nephrons from the same region as the reconstructed ventral nephron ranged between 110 × 70 μm and 100 × 80 μm. The average height of the Malpighian corpuscles was 102 ± 2 μm (n = 7) and the average width was 71 ± 4 μm (n = 5). In the segmental divisions of the most fron-

Fig. 2. Graphic representation of the urogenital system in female *Geotrypetes seraphini*.

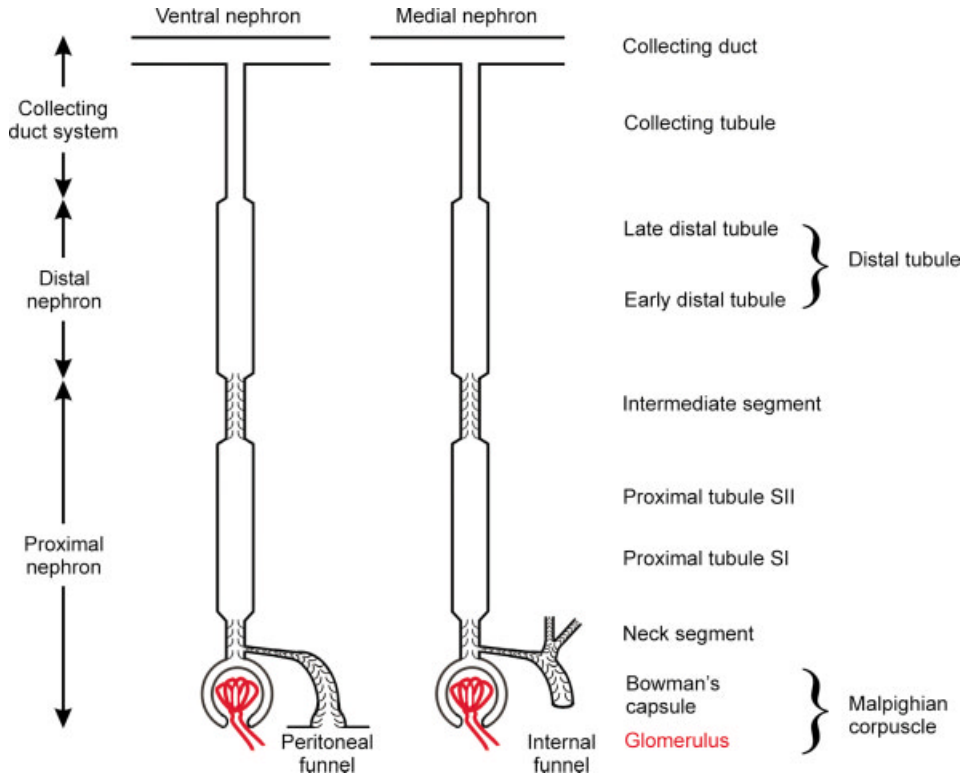


Fig. 3. Schematic representation of nephron types in the kidney of *Geotrypetes seraphini*. From the ventral surface of the kidney, peritoneal funnels connect to the neck segment of ventral nephrons through a ciliated tubule. Medial nephrons lack this connection to the coelom. In these nephrons a ciliated tubule also leaves the neck segment, but the tubule ends blindly or connects to an internal blind funnel, often linking 3–4 Malpighian corpuscles. Both ventral and medial nephrons are composed of a filtration unit, the Malpighian corpuscle, and a renal tubule, which in the midfrontal to caudal part of the kidney can be divided into six morphologically distinct segments: neck segment, proximal tubule, intermediate segment, early distal tubule, late distal tubule, and collecting tubule. Collecting tubules merge and open into collecting ducts. Not shown: In ventral nephrons the efferent arterioles often have a smaller diameter than the afferent arteriole, while there are no differences in medial nephron arteriolar size. In medial nephrons the efferent arterioles exit at approximately the same point, creating a vascular pole, while efferent arterioles of ventral nephrons often leave the corpuscles more dorsally.

tal part of the kidney, Malpighian corpuscles were larger (Fig. 6B,C). In this region maximal dimensions of 140 × 90 μm were recorded from a medial nephron. In Specimen 3, which was kept under aquatic conditions, much larger dimensions were observed. From a region of the middle part of the left kidney, just posterior to the ovaries, an average height of 153 ± 8 μm (n = 6) and a width of 97 ± 2 μm (n = 6) were measured with the maximal dimension from a medial nephron of 180 × 100 μm.

A rather straight ciliated neck segment with an outer diameter of 15–25 μm and a length of 300 μm proceeds in a lateral direction from the urinary pole of the Malpighian corpuscle and joins the proximal tubule in the lateral part of the dorsolateral zone (Fig. 5B). At a position on the neck segment 190 μm from the urinary pole, a ciliated tubule, 110 μm long, connects the nephron to the coelom through a ciliated peritoneal funnel (Fig. 5A). This funnel has a length of 130 μm and opening dimensions of 55 × 75 μm. The intensely convoluted proximal tubule is, at 2,025 μm, the longest segment of the renal tubule (Fig. 5C). The tubule's outer diameter increases from ~35 μm at the transition zone with the neck segment to 40–55 μm. From the point of connection with the neck segment the tubule runs in a dorsal direction, where it undergoes considerable convolution. A somewhat straighter part connects these convolutions with another set of convolutions at the level of the Malpighian corpuscle, and the proximal tubule finally opens into a 340 μm long ciliated intermediate segment in the ventromedial zone. This straight segment has an outer diameter of 15–25 μm and it runs in a cranial direction and toward the medial margin of the kidney where it connects to an 870 μm long distal tubule. A gradual increase in outer tubule diameter to 30–35 μm is seen following emersion from the intermediate segment. Approximately half of the distal tubule length is accounted for by a set of heavy convolutions in the ventromedial zone. The distal tubule comes into close contact with the filtration unit before it increases in diameter to 35–40 μm and leaves the ventromedial zone and runs into the dorsolateral zone where it turns and runs back. The distal tubule makes another set of convolutions in the ventrome-

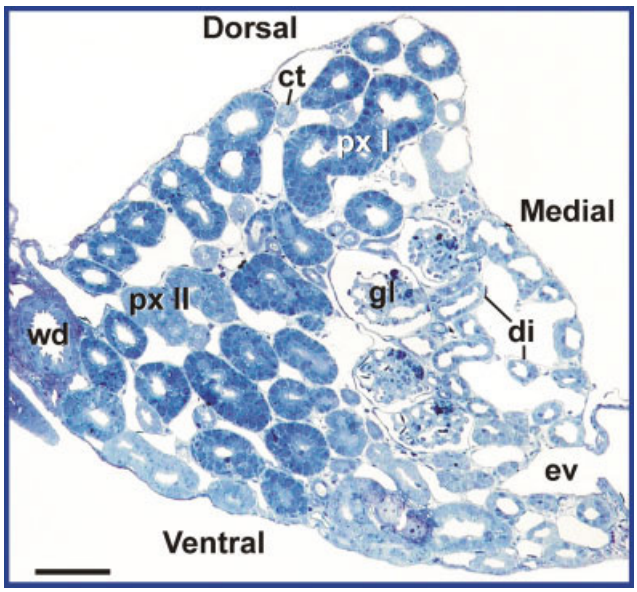


Fig. 4. *Geotrypetes seraphini*. Transverse sections of the left kidney from Specimen 3 at a level corresponding to the rear edge of the ovaries (see Fig. 2). Araldite section, 2 μm, stained with toluidine blue. LM. When viewed in transverse sections the kidney can be divided into a ventromedial zone and a dorsolateral zone. The ventromedial zone is characterized by the presence of Malpighian corpuscles, distal tubules, and peritoneal funnels with their ciliated tubules, along with the neck and intermediate segments of ventral nephrons. The dorsolateral zone is dominated by the presence of proximal tubules and tubules of the collecting duct system. ct, collecting tubule; di, distal tubules; ev, efferent renal vein; gl, glomerulus; px I, proximal tubule segment I; px II, proximal tubule segment II; wd, Wolffian duct. Scale bar = 100 μm.

dial zone before merging with a 310 μm long collecting tubule. Toward the collecting tubule the outer tubule diameter decreases to 15–20 μm. The first set of distal tubule convolutions clearly shows characteristics of the amphibian early distal tubule (Fig. 5D). A clear division into early and late segments is, however, difficult in this ventral nephron, although the gradual increase in outer tubule diameter in the second half of the tubule is accompanied by a more rounded appearance of the nuclei (Fig. 5E). From the junction with the distal tubule in the ventromedial zone, the collecting tubule (Fig. 5F) runs in a laterocaudal direction and connects the nephron with the branches of the collecting duct system. The collecting tubule has an outer tubule diameter of 15–20 μm.

Malpighian Corpuscle (Figs. 3, 6, 7)

The following description of Malpighian corpuscle ultrastructure is mainly based on ultrathin sections from the aquatic specimen and from the frontal segmental divisions of terrestrial specimens where Malpighian corpuscle diameters were largest.

The Malpighian corpuscle is formed by two structures—the vascular loops of the glomerulus and the capsule of Bowman surrounding the glomerulus (Fig. 7A). The capsule of Bowman consists of a visceral layer composed of podocytes, which encircle the glomerular capillaries, and a thin parietal layer, which is continuous with the epithelium of the renal tubule. The space between the visceral and parietal layers, the nephrocoel or urinary space, is continuous with the lumen of the tubule (Fig. 6C).

The epithelium of the parietal layer is composed of polygonally shaped squamous cells. The perikaryon of these cells has a height of 2–3 μm and bulges slightly into the urinary space (Fig. 7B). A small number of scattered microvilli covers the apical surface of the cells and a single apical cilium is occasionally seen (not shown). The cytoplasm moreover contains the usual set of cell organelles. A number of small vesicles are present beneath the apical cell membrane (not shown).

The podocyte epithelium of the capsule's visceral layer consists of cell bodies containing the nucleus and interdigitating cell processes that send out foot processes (Fig. 7A,C,D). These alternating foot processes rest on the 0.2–0.6-μm thick basement membrane and form narrow filtration slits, which are bridged by diaphragms (Fig. 7C,D). Mitochondria are present in the perinuclear area and in the cell processes, along with microfilaments and microtubules (Fig. 7C). The cytoplasm, moreover, contains a Golgi complex, endoplasmic reticulum, polyribosomes, vesicles, lipid droplets, and a varying degree of electron-dense granules. The podocytes are covered by a glycocalyx and exhibit microvilli.

The three-layered basement membrane consists of a lamina lucida beneath the podocyte processes, a lamina densa (basal lamina), and a lamina fibroreticularis bordering the endothelium of the glomerular capillaries. Lamina lucida and lamina densa together have a constant thickness of 75–85 nm (Fig. 7C,D).

The glomerular capillaries consist of an endothelium with fenestrae of varying sizes (100–300 nm). The endothelial cell bodies, containing nuclei, bulge into the capillary lumen (Fig. 7D). A smaller number of mesangial cells are found on the endothelial side of the basement membrane (Fig. 7A). These cells occasionally send out processes into the lamina fibroreticularis (Fig. 7C,D).

The filtration barrier is thus constituted by the fenestrated endothelium of the glomerular capillaries, the glomerular basement membrane, and the foot processes of the podocytes. Primary urine is formed by ultrafiltration of blood from the glomerular capillaries across this barrier and into the nephrocoel. The area and permeability of the filtration barrier may be regulated by the presence of cellular processes from the mesangial cells.

Renal Tubule (Figs. 2, 4, 6)

In the terrestrial specimens (Specimens 1 and 2) the lumina of the renal tubules were often collapsed

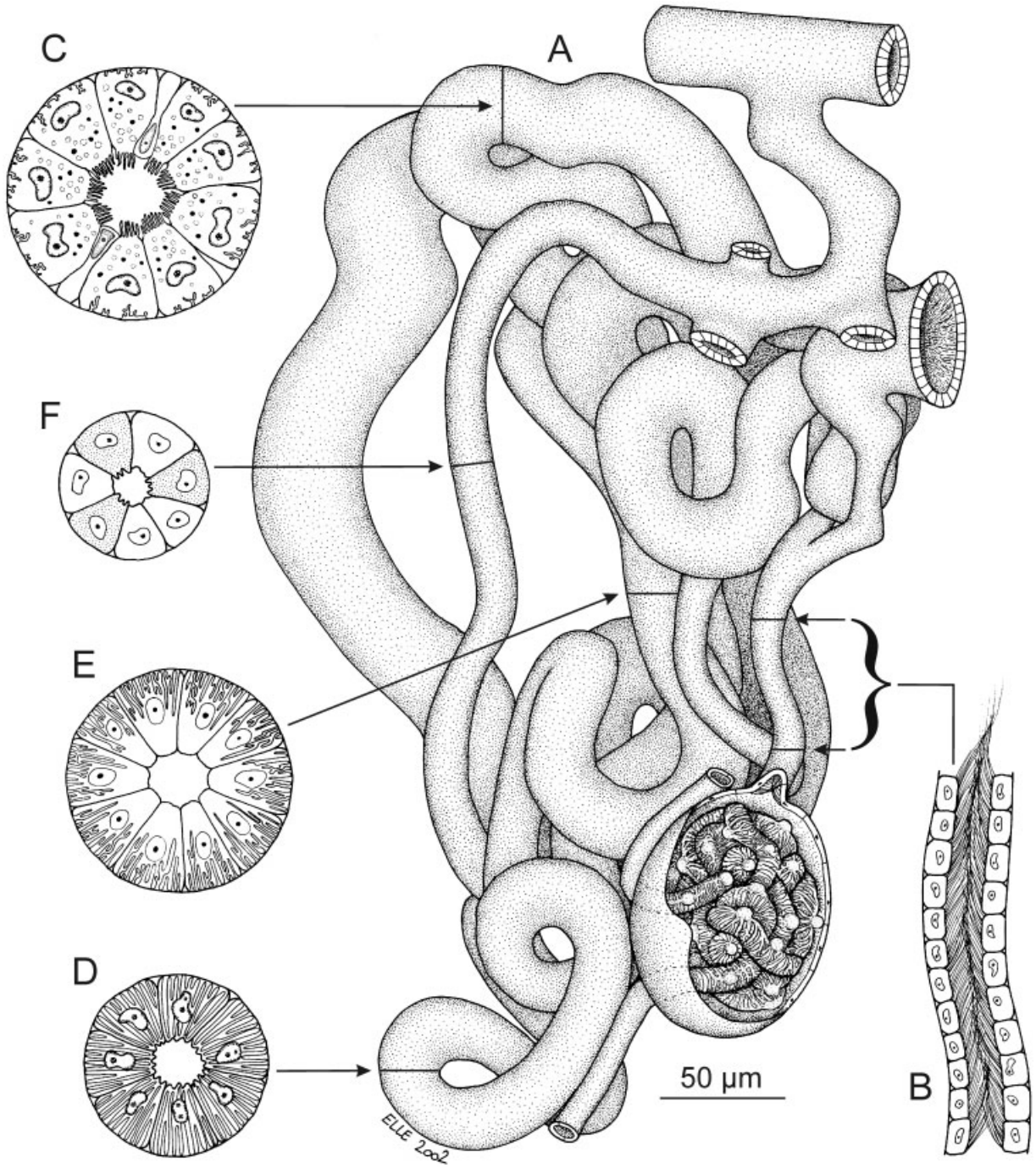


Fig. 5. *Geotrypetes seraphini.* Graphic reconstruction of a ventral nephron from the midfrontal region of the left kidney from the 16-cm long Specimen 1 (**A**). The nephron is viewed from its caudal edge. Up is lateral and down is medial. Dorsal is left and ventral is right. A longitudinal section is shown of the neck segment (**B**) and transverse sections are shown of the proximal tubule segment I (**C**), the early distal tubule (**D**), the late distal tubule (**E**) and the collecting tubule (**F**). The collecting tubule connects the nephron with the branches of the collecting duct system, which finally open into the Wolffian duct.

(Fig. 6C–F). This was not the case for the aquatic specimen (Specimen 3) in which the renal tubules therefore have greater outer diameters (Figs. 4, 6A). The greater diameters are most pronounced for the proximal and collecting tubules. In Specimen 3, proximal and collecting tubule outer diameters are 25–30% and 50–60% larger, ranging from 50–70 µm and 25–30 µm, respectively. In spite of the collapsed lumens in Specimens 1 and 2, there are no significant differences in renal tubule cell ultrastructure between kidneys of animals kept either terrestrially or aquatically. Sections from the seg-

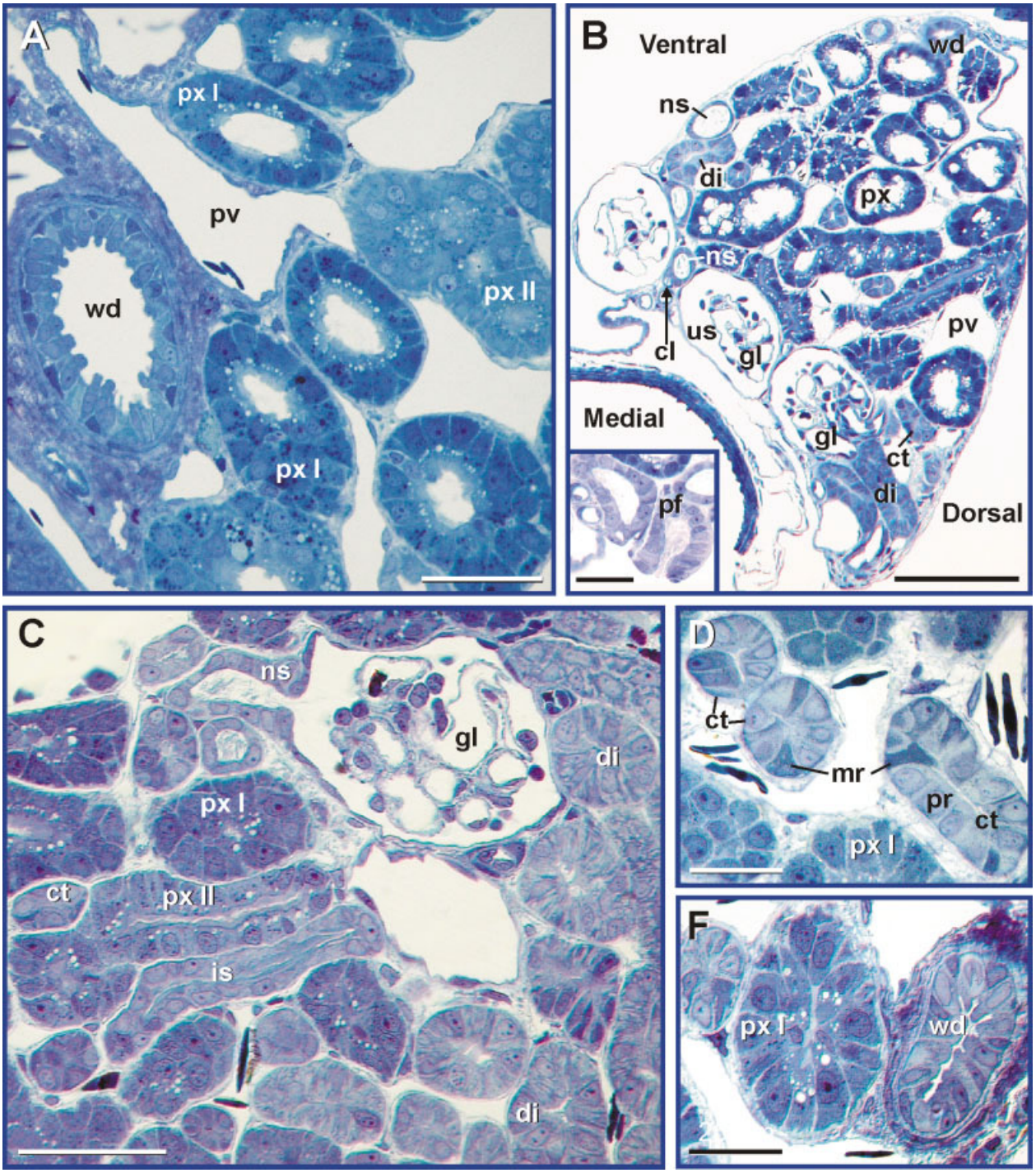


Fig. 6. *Geotrypetes seraphini.* Araldite sections, 2 μm, stained with toluidine blue. LM. **A:** Transverse section from the dorsolateral zone of the left kidney from Specimen 3 showing proximal tubules and the Wolffian duct. **B:** Transverse section from frontal segmental division of the left kidney from Specimen 1. The section is from the division shown in Figure 1B. Note the large Malpighian corpuscles and the irregularity in size and shape of the proximal tubule cells. Insert: peritoneal funnel opening into the coelom (pf). To the left of this funnel a blind funnel, which does not connect to the coelom, is seen. **C–F:** Transverse sections obtained from the left kidney of Specimen 1 at the level of the ovaries. In the kidney regions caudal to the segmental divisions of this specimen the lumen of the renal tubules was often collapsed. cl, ciliated tubule; ct, collecting tubule; di, distal tubule; gl, glomerulus; is, intermediate segment; mr, mitochondria-rich cell; ns, neck segment; pf, peritoneal funnel; pr, principal cell; pv, peritubular vessel; px I, proximal tubule segment I; px II, proximal tubule segment II; us, urinary space; wd, Wolffian duct. Scale bars = 50 μm (**A**), 100 μm (**B**), 50 μm (**C**), 25 μm (**D,F**).

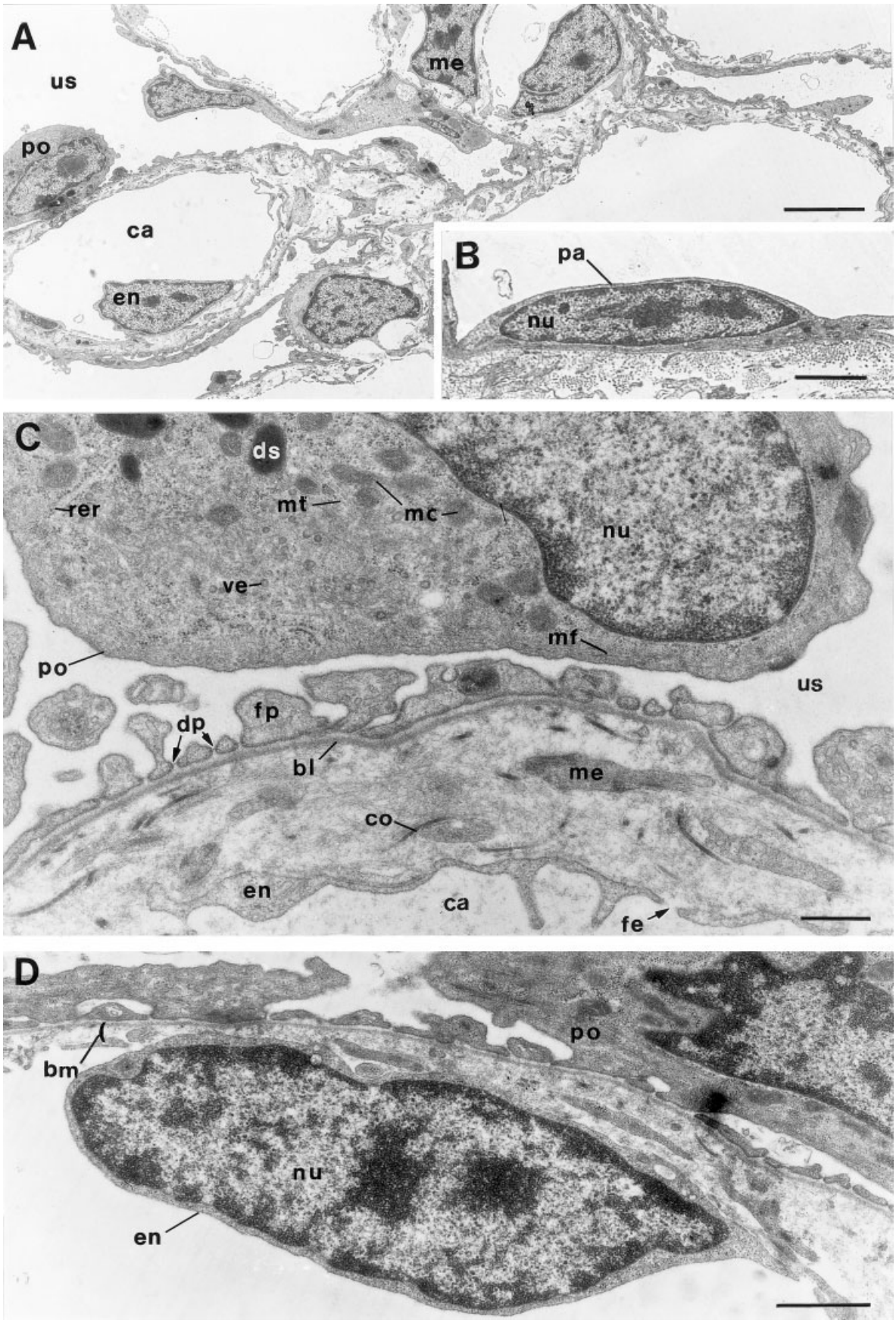


Fig. 7. *Geotrypetes seraphini.* Malpighian corpuscle. TEM. **A:** Vascular loops of the glomerulus. **B:** Parietal cell. **C,D:** Filtration barrier between the urinary and capillary space. bl, basal lamina; bm, basement membrane; ca, capillary space; co, collagen; dp, diaphragms; ds, dense granules; en, endothelial cell; fe, fenestration; fp, foot process; mc, mitochondria; me, mesangial cell; mf, microfilaments; mt, microtubules; nu, nucleus; po, podocyte; rer, rough endoplasmic reticulum; us, urinary space; ve, vesicle. Scale bars = 5 μm (**A**), 2 μm (**B**), 0.5 μm (**C**), and 1 μm (**D**).

mental divisions of the most anterior part of the kidney in Specimen 1 (Fig. 2), however, showed that proximal tubule cytology in this frontal region of the kidney differs from that in the more caudal regions (Fig. 6B). In the following section, we describe the ultrastructure of the different renal tubule segments based on sections from the three specimens.

Neck and Intermediate Segments (Figs. 3, 6C, 8)

Two ciliated segments are present in the renal tubule, i.e., the neck segment and the intermediate segment (Figs. 3, 6C). The neck segment is the first part of the renal tubule and it is continuous with the parietal layer of the renal corpuscle. It connects the corpuscle with the proximal tubule, whereas the intermediate segment connects the proximal and distal tubules. The epithelium of these multiciliated segments consists of 7–8 μm high cuboidal cells with centrally located nuclei (Fig. 8). A few minute microvilli cover the apical surface of the cells (Fig. 8A,C). The cilia have the conventional 9 + 2 microtubular arrangement and the apical cytoplasm is characterized by the presence of basal bodies, striated rootlets, and a terminal web (Fig. 8B,D). Many mitochondria, together with other typical complements of cell organelles, are found in the cytoplasm. The lateral cell membranes form interdigitating microvillar projections, whereas the basal cell membranes lack infoldings (Fig. 8A,C).

Proximal Tubule (Figs. 1–3, 5, 6, 9–11)

An abrupt change in tubule diameter and cell morphology characterizes the transition from the neck segment to the following segment, the proximal tubule. The proximal tubule is heterocellular, composed of proximal tubule principal cells and a minority cell type, the so-called "bald-headed cells" (Fig. 9). Bald-headed cells appear as single intercalated cells between the principal cells. The apical surfaces of principal cells carry microvilli, whereas bald-headed cells, as their name implies, have a rather smooth apical surface. Three morphologically different proximal tubule segments can be recognized in the *Geotrypetes* kidney.

In the segmental divisions of the frontal part of the kidney (Figs. 1, 2), the proximal tubule principal cells are variable in size and shape and have a moderately developed brush border (Figs. 6B, 9A). The epithelium of this frontal region has a height of 3–10 μm and appears the same throughout the tubule. A few tubules with this characteristic principal cell form are also present in the region of the nephron reconstructed in Figure 5, where they were the only proximal tubules to have a lumen. Proximal tubules with similar cytology were not found in the more caudal part of the kidney.

In the "kidney proper" the proximal tubule can be divided into two distinct segments (Figs. 3, 6A,C, 9B, 10, 11). The first part of the proximal tubule, proximal tubule segment I, connects to the neck segment. The transition from the neck segment is characterized by an increase in cell height to 15–20 μm and the appearance of a regular, 3–5 μm high, brush border (Figs. 6A,C,F, 9B). In the more distal part of the proximal tubule, proximal tubule segment II, the cells decrease in height and the principal cells appear less osmiophilic (Figs. 6A,C, 11). Proximal tubule segment II connects to the intermediate segment. The following description of proximal tubule cytology is based primarily on segment I. The deviations from this pattern, which characterize segment II and the proximal tubule found in the frontal kidney divisions, are presented at the end of the paragraph.

The microvilli of principal cells contain microfilaments that extend into the apical cytoplasmic zone (Fig. 10B), which is dominated by a prominent endocytotic-lysosomal apparatus: apical cytoplasmic invaginations, vesicles, vacuoles, tubular inclusions, and microtubules fill the apical cytoplasm and lysosomes of varying size appear below the apical zone (Figs. 9–11). A large and regular nucleus with a distinct nucleolus is situated centrally in the cell (Figs. 9–11). Numerous, often elongate, mitochondria are found in the perinuclear zone along with a Golgi complex, rough endoplasmic reticulum, and large amounts of smooth endoplasmic reticulum (Figs. 9–11). Dilated lateral intercellular spaces contain microvillar extensions of the lateral membrane. The intercellular spaces are narrow beneath the apical junctional complex and become wider toward the base of the cells, where they are continuous with an extracellular labyrinth formed by the basal cell membrane (Figs. 9B, 10A,C). Giant principal cells with double nuclei are found occasionally (Fig. 10A). A single cilium extends from the apical region of the cell into the tubule lumen (not shown).

The bald-headed cells have an hourglass form and are smaller than the principal cells between which they are intercalated (Fig. 9). The apical and basal cell membranes of these cells appear smooth, while the lateral cell membrane occasionally sends out small microvillar projections into the lateral intercellular space. The nucleus is most often situated in the apical cytoplasm (Fig. 9). The cytoplasm has a pale appearance and contains small elongate mitochondria, polyribosomes, a Golgi complex, and endoplasmic reticulum (Figs. 9, 10B). Numerous small vesicles are often found in the apical cytoplasm (not shown).

The principal cells of proximal tubule segment II are characterized by a less dense cytoplasm and appear lighter, especially when studied under the light microscope (Fig. 6A,C). In all three specimens, segment II has a very narrow or nonvisible lumen (Fig. 6A,C). The cells of this segment are addition-

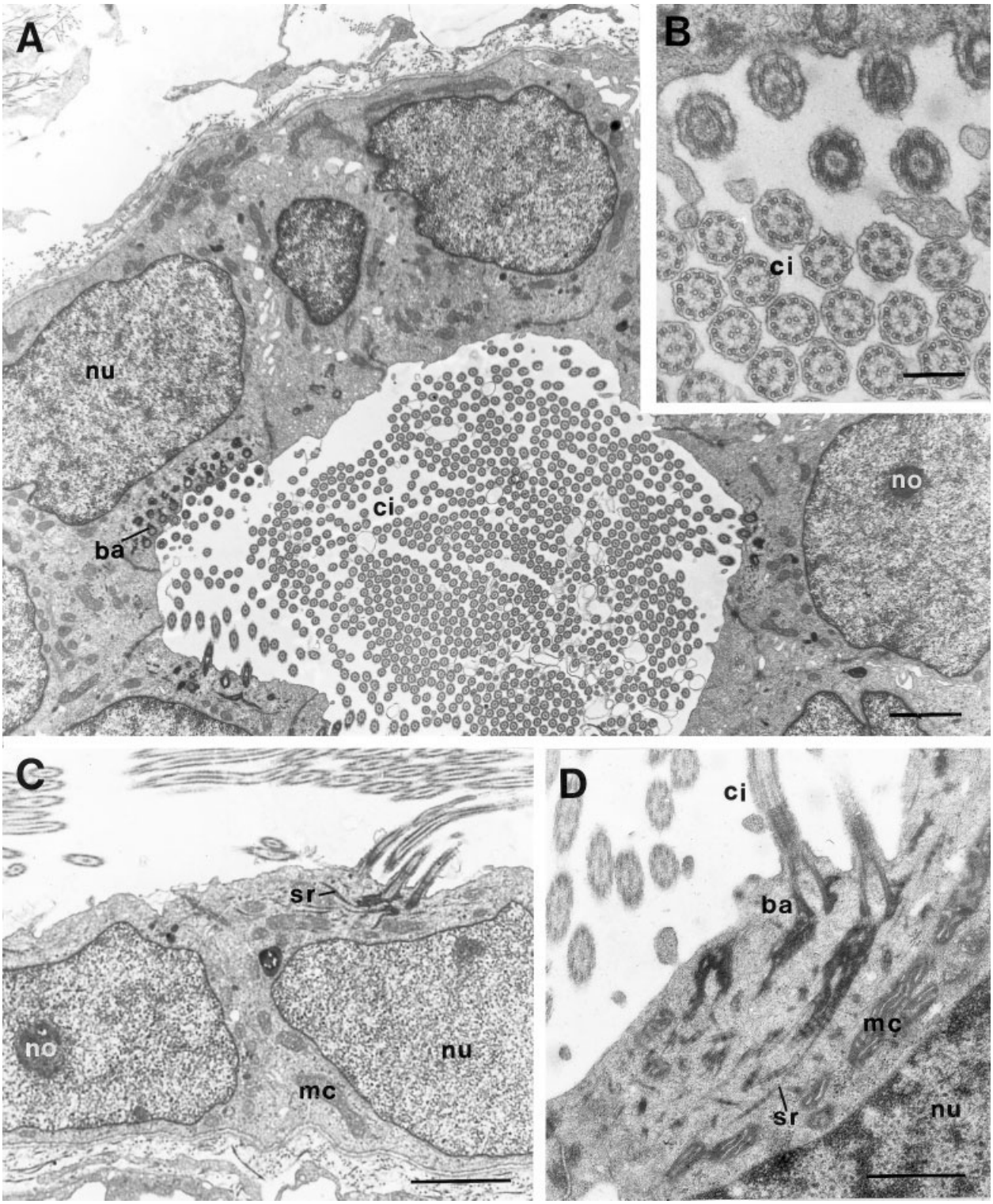


Fig. 8. *Geotrypetes seraphini.* Neck and intermediate segments. TEM. **A,B:** Transverse section and close-up of the ciliated neck segment. **C,D:** Intermediate segment. ba, basal body; ci, cilia; mc, mitochondria; no, nucleolus; nu, nucleus; sr, striated rootlets. Scale bars = 2 μm (**A,C**), 0.25 μm (**B**), and 1 μm (**D**).

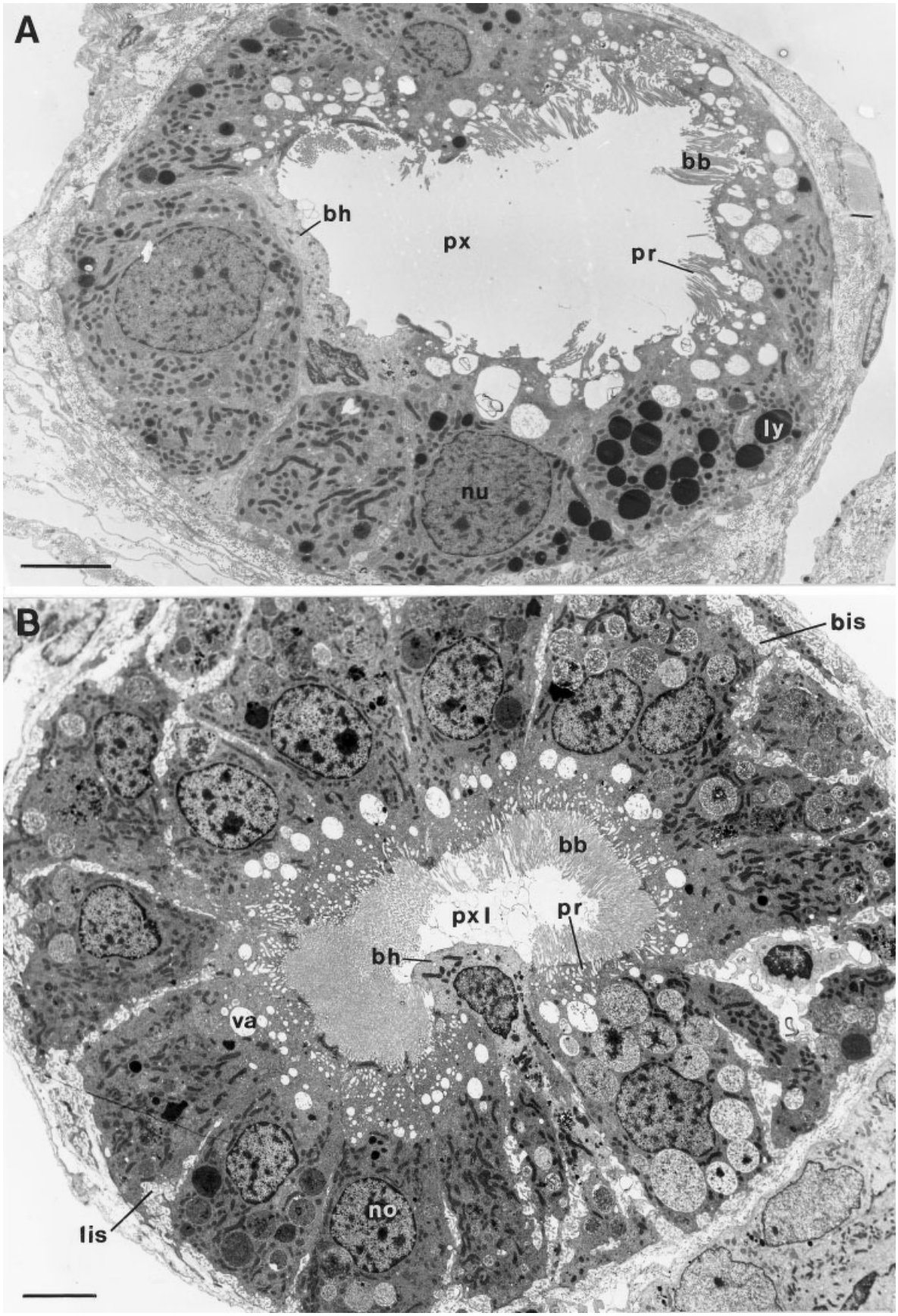


Fig. 9. *Geotrypetes seraphini.* Proximal tubule. TEM. **A:** Transverse section through a proximal tubule (px) from one of the frontal divisions of the kidney. **B:** From the caudal part of the kidney, segment I (px I). bb, brush border; bh, bald-headed cells; bis, basal intercellular space; lis, lateral intercellular space; ly, lysosome; no, nucleolus; nu, nucleus; pr, principal cells; va, vacuole. Scale bars = 5 μm.

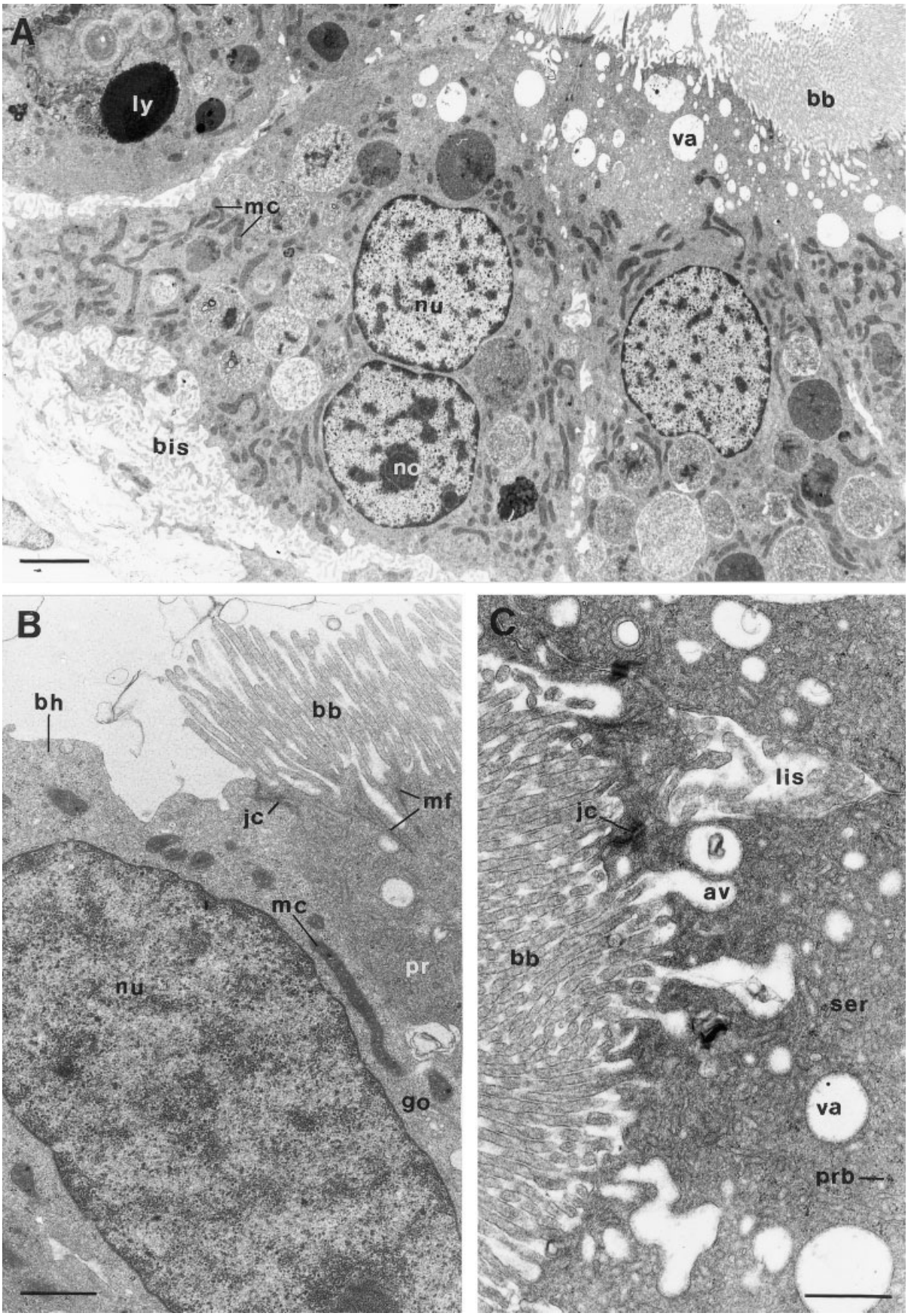


Fig. 10. *Geotrypetes seraphini.* Proximal tubule segment I. TEM. **A:** Epithelial principal cell with double nuclei. **B:** Close-up of the luminal border between a bald-headed cell (bh) and a principal cell (pr). **C:** Close-up of the apical cytoplasm of a proximal tubule principal cell. av, apical invagination; bb, brush border; bis, basal intercellular space; go, Golgi apparatus; jc, junctional complexes; ly, lysosome; mc, mitochondria; mf, microfilaments; no, nucleolus; nu, nuclei; prb, polyribosomes; ser, smooth endoplasmic reticulum; va, vacuoles. Scale bars = 3 μm (**A**) and 1 μm (**B,C**).

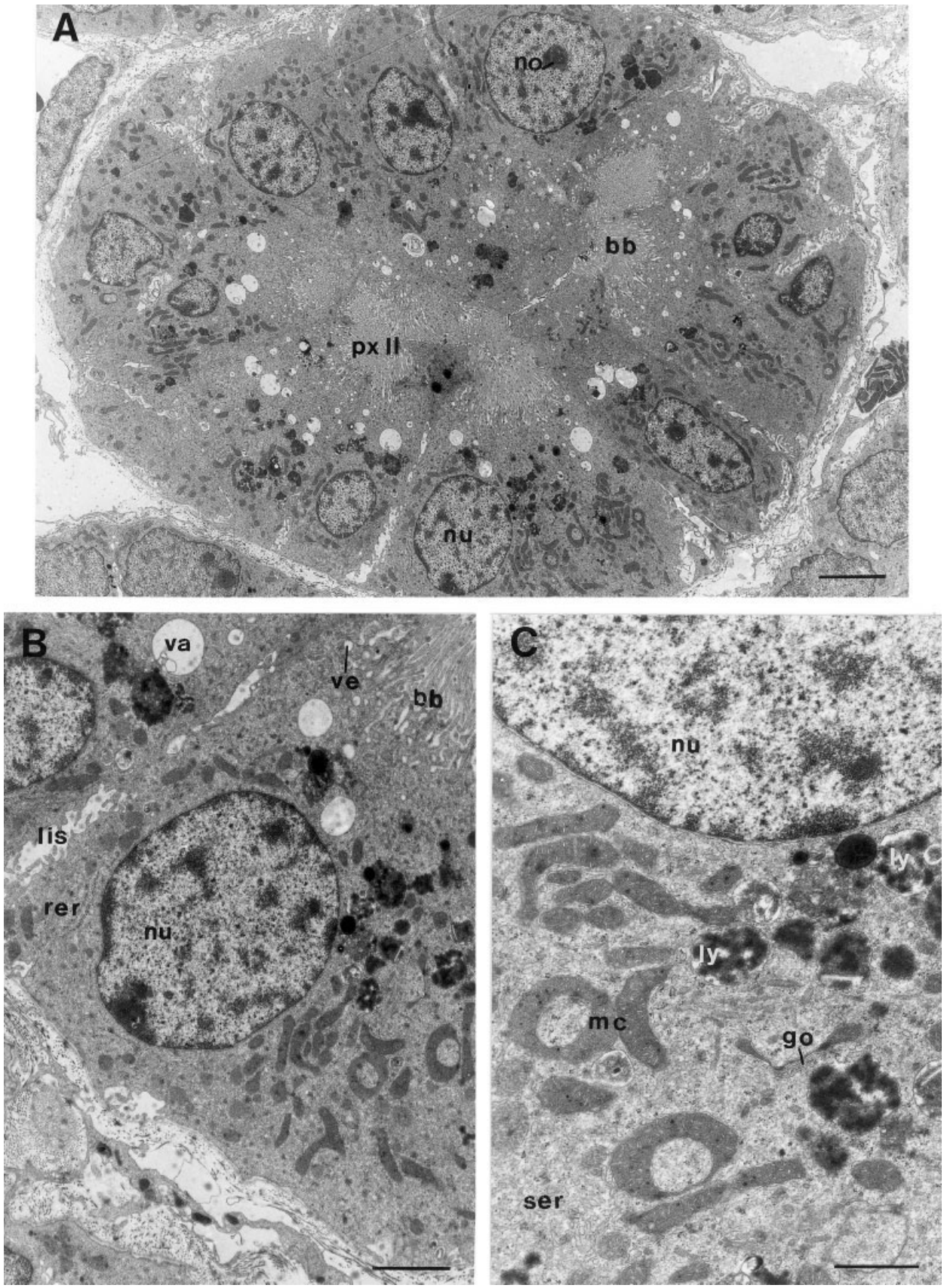


Fig. 11. *Geotrypetes seraphini*. Proximal tubule, segment II (px II). TEM. **A:** Transverse section of tubule with a characteristic narrow lumen. **B,C:** Close-ups of principal cells. bb, brush border; go, Golgi apparatus; lis, lateral intercellular space; ly, lysosomes; mc, mitochondria nu, nuclei; rer, rough endoplasmic reticulum; ser, smooth endoplasmic reticulum, va, vacuole; ve, vesicle. Scale bars = 5 μm (**A**), 2 μm (**B**), and 1 μm (**C**).

ally distinguished by a less developed basal labyrinth and mitochondria of many different shapes (Fig. 11).

The principal cells of the proximal tubule found in the frontal divisions of the kidney are easily recognized by the variability and irregularity of their size and shape. The cytoplasm of these proximal tubule cells is, like that of the more caudal tubules, characterized by a conspicuous endocytotic-lysosomal apparatus. The brush border is, however, irregular and only moderately developed (Figs. 6B, 9A). Baldheaded cells have an hourglass form as in the more caudal proximal tubules.

Distal Tubule (Figs. 3, 5, 6, 12, 13)

The distal tubule can be subdivided into early and late portions according to differences in ultrastructure of the epithelial cells (Figs. 3, 5). For discussions of distal tubule terminology, see Hentschel and Elger (1989) and Møbjerg et al. (1998). This subdivision is most obvious in medial nephrons of the midfrontal to caudal part of the kidney and is only clearly recognizable at the electron microscopic level (Figs. 6C, 12, 13).

Early Distal Tubule (Figs. 6, 12, 13)

An abrupt change of the epithelium from the intermediate segment marks the beginning of the distal tubule. The cuboidal epithelial cells of the first part of the distal tubule, the early distal tubule, are 7–8 μm high and have a centrally located lobulate nucleus with relatively large amounts of heterochromatin (Fig. 12A,B). The early distal tubule cells interdigitate with each other via conspicuous infoldings of the lateral and basal cell membranes (Figs. 12B, 13A,B). The lateral interdigitations reach and often include the apical cell membranes, causing the appearance of a great number of apical cell junctions (Fig. 13A). The apical surface of the cells has many small and irregular microvilli containing microfilaments (Fig. 13A). Many, often vertically arranged, mitochondria are associated with the basolateral cell membrane infoldings (Figs. 12A,B, 13A,B). A narrow area of apical cytoplasm contains spherical or elongated vesicles and multivesicular bodies (Fig. 13A,B). The cytoplasm, moreover, contains typical cell organelles such as microtubules, lipid droplets, and lysosomes of different shapes, a Golgi complex, endoplasmic reticulum and polyribosomes. A single cilium is often found extending into the lumen of the tubule (not shown). The early distal tubule comes into close contact with the nephron's filtration unit (Fig. 6B). However, no specialized epithelial structures are apparent at the tubule attachment site.

Late Distal Tubule (Figs. 12, 13)

The 12–14 μm high cells forming the late distal tubule's epithelium are characterized by the presence of ovoid nuclei with small amounts of heterochromatin and a distinct nucleolus (Figs. 12A,C, 13C,D). This epithelium corresponds to the late distal tubule section I as described for the toad, *Bufo bufo* (see Møbjerg et al., 1998). The cells have well-developed lateral and basal cell membrane infoldings, but these are less pronounced than in the early distal tubule and they do not include the apical cell membrane (Fig. 12C). Mitochondria are found in the perinuclear zone and are vertically arranged between the membrane infoldings (Figs. 12A,C, 13C,D). The apical surface has short, thick, irregular microvilli (Fig. 12C). The microvilli are especially numerous in the junctional areas (Fig. 13C). Vesicles and multivesicular bodies are seen in the apical cytoplasm (Figs. 12C, 13C,D). The cytoplasm, moreover, contains a Golgi complex, endoplasmic reticulum, polyribosomes, microtubules, microfilaments, lipid droplets, and lysosomes. A single cilium extends from the apical region of the cell into the tubule lumen (not shown).

Occasionally, cells with dark cytoplasm and osmiophobic mitochondria are found intercalated between the principal cells (Fig. 13C). These cells are morphologically different from the intercalated or mitochondria-rich cells of the collecting duct system. Towards the collecting tubule the cells of the late distal tubule decrease in height and have fewer membrane infoldings.

Collecting Duct System (Figs. 3, 5, 14)

The collecting duct system comprises collecting tubules and collecting ducts. The collecting tubule is the first part of the collecting duct system, which is heterocellular and is characterized by the presence of two cell types: principal cells and a minority cell type, the darker stained intercalated or mitochondria-rich cells (Figs. 3, 5, 14). For discussions of terminology, see Hentschel and Elger (1989) and Møbjerg et al. (1998).

The apical surface of principal cells bears a few microvilli (Fig. 14A–C). Within the cytoplasm of these cells mitochondria, smooth and rough endoplasmic reticulum, a Golgi complex, microtubules, and lipid droplets are seen (Fig. 14). Mitochondria-rich cells are characterized by their abundant mitochondria and dense cytoplasm (Fig. 14A,C). The apical cytoplasm contains many small vesicles and the apical surface is amplified by microvilli and microplicae (Fig. 14A,C,D). The cytoplasm also contains a Golgi complex, endoplasmic reticulum, polyribosomes, and lysosomes.

The collecting tubules are formed by cuboidal cells with a height of 7–8 μm (Fig. 14A,B). The cells increase in height to 9–10 μm toward the transition to the collecting ducts, which have a cell height of 10–13 μm (Fig. 14C). In the collecting tubule both cell types have centrally situated nuclei, which are slightly heterochromatic and irregular (Fig. 14A,B).

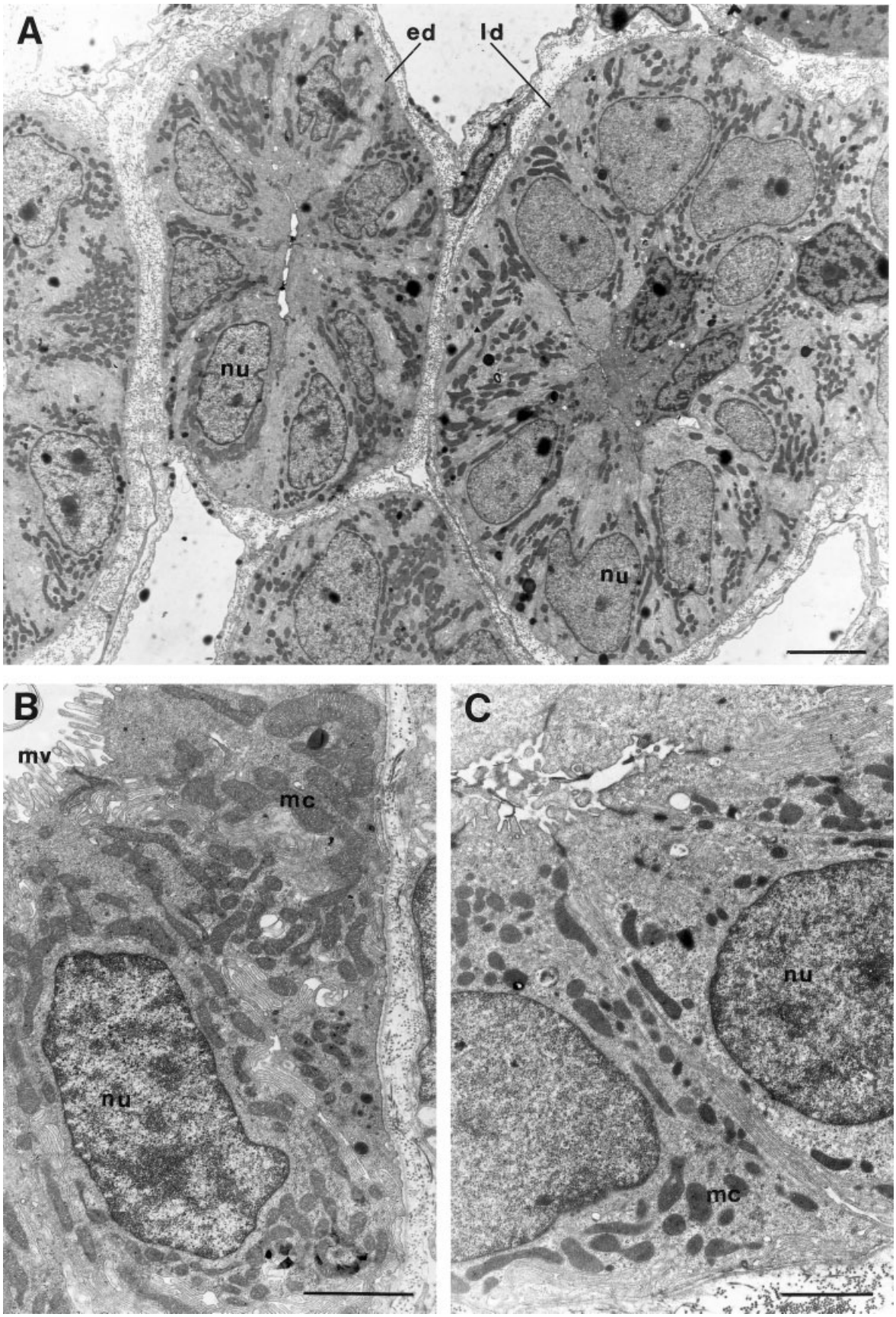


Fig. 12. *Geotrypetes seraphini.* Distal tubule. TEM. **A:** Transverse sections of early distal tubule (ed) and late distal tubule (ld). **B:** Epithelial cell from early distal tubule. **C:** Epithelial cells from late distal tubule. mc, mitochondria; mv, microvilli; nu, nuclei. Scale bars = 5 μm (**A**), 2 μm (**B,C**).

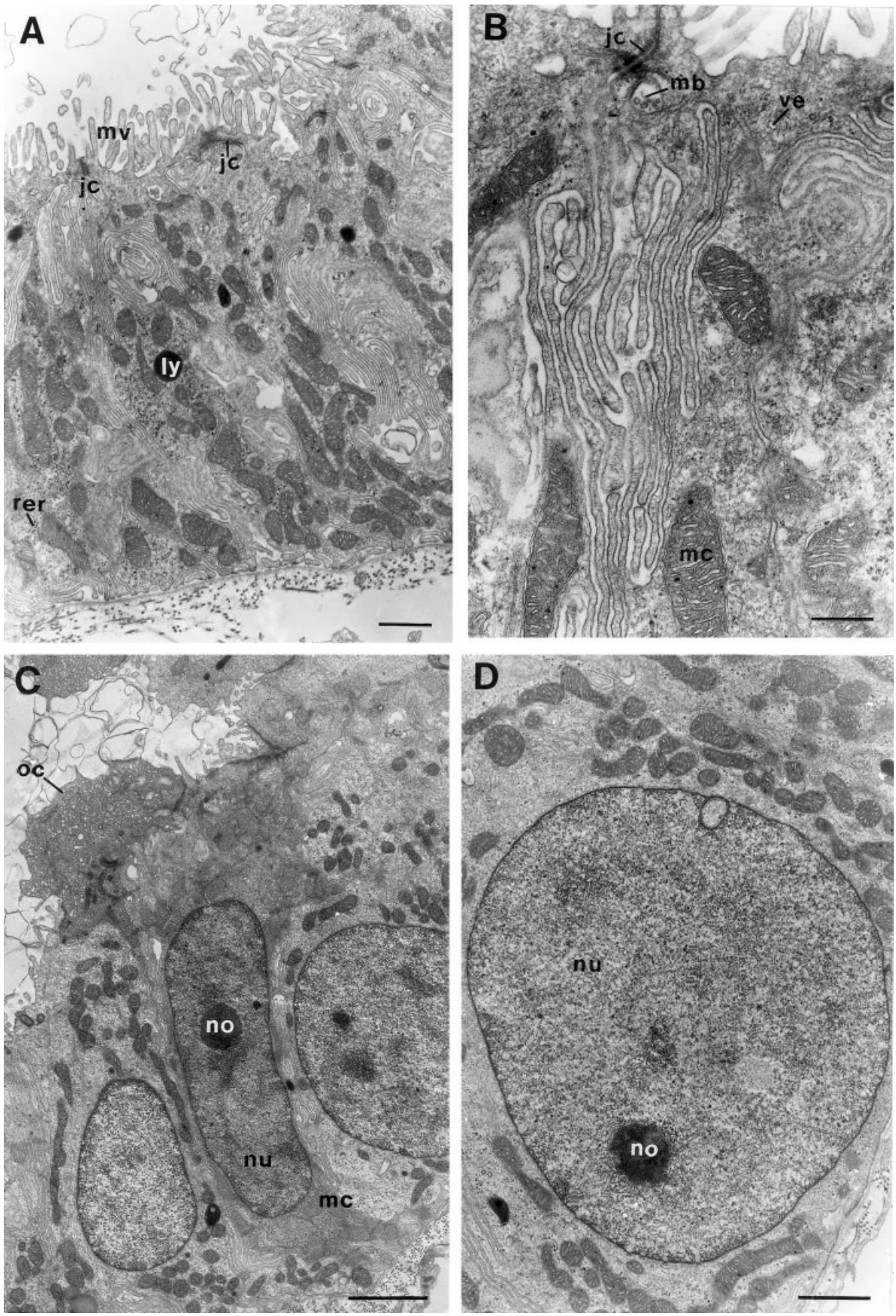


Fig. 13. *Geotrypetes seraphini*. Early distal tubule (**A,B**) and late distal tubule **(C,D**). TEM. **A:** Early distal tubule epithelium with pronounced infoldings of the basolateral cell membranes. **B:** Close-up of the apical part of the early distal epithelium. **C:** Late distal tubule osmiophilic cell (oc) interposed between principal cells. Note the dark cytoplasm and osmiophobic mitochondria. **D:** Close-up of late distal tubule cell with large ovoid nucleus and less pronounced infoldings of the cellular membrane. jc, junctional complexes; ly, lysosome; mb, multivesicular body; mc, mitochondria; mv, microvilli; no, nucleoli; nu, nuclei; rer, rough endoplasmic reticulum; ve, vesicle. Scale bars = 1 μm (**A**), 5 μm (**B**), 3 μm (**C**), and 1 μm (**D**).

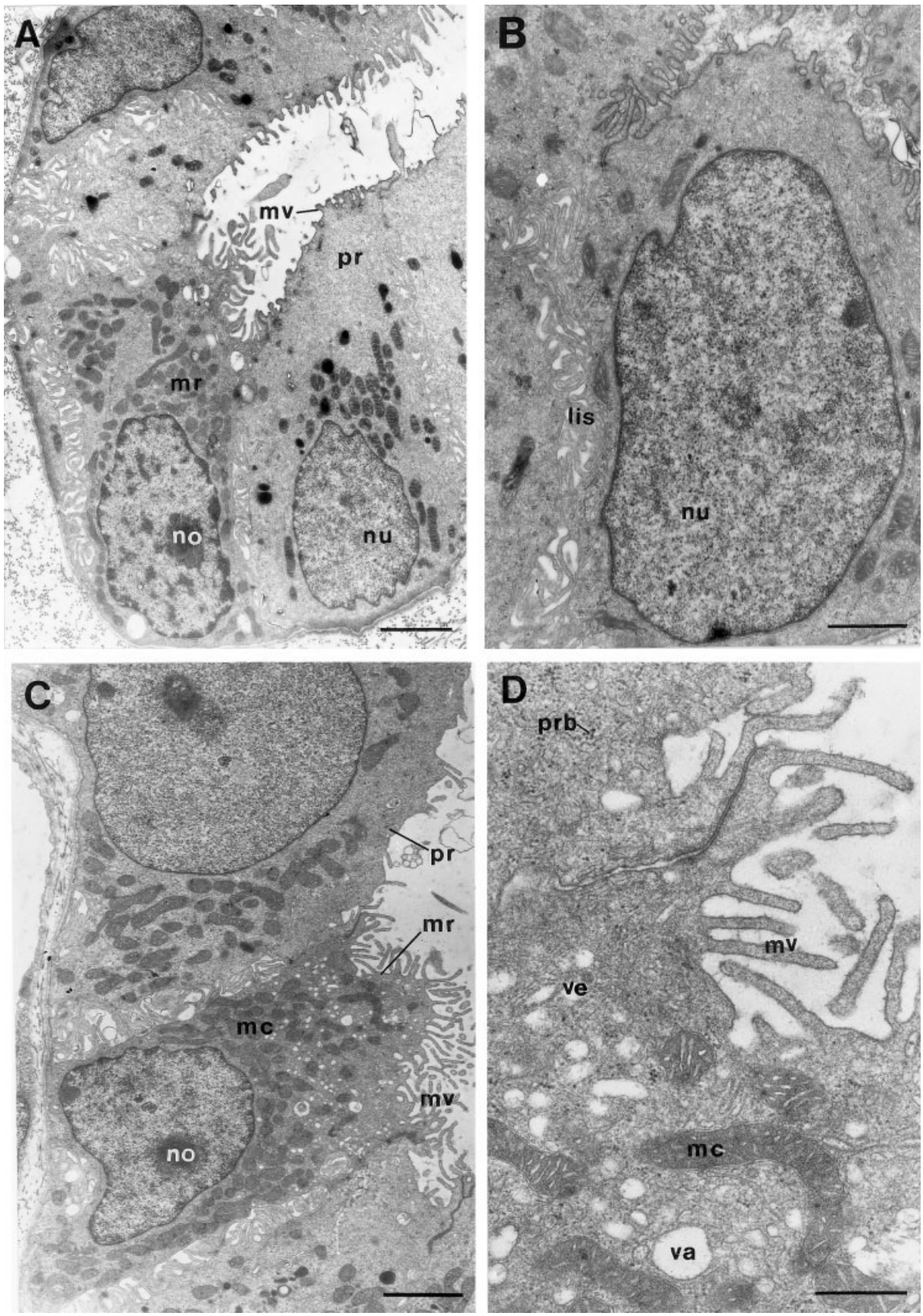


Fig. 14. *Geotrypes seraphini.* Collecting duct system. TEM. **A:** Transverse section of the heterocellular collecting tubule with intercalated mitochondria-rich cell (mr) and principal cells (pr). **B:** Collecting tubule principal cell. **C:** Collecting duct formed by mitochondria-rich (mr) and principal (pr) cells. **D:** Close-up of the apical part of an intercalated cell at the region of its junction with a principal cell. Note the deep tight junction between the two cells. lis, lateral intercellular space; mc, mitochondria; mv, microvilli; no, nucleoli; nu, nuclei, prb, polyribosomes; va, vacuole; ve, vesicle. Scale bars = 3 μm (**A**), 1 μm (**B**), 2 μm (**C**) and 0.5 μm (**D**).

Cells forming the ducts have centrally situated ovoid nuclei with small amounts of heterochromatin (Fig. 14C). In the collecting tubules amplifications of the cell membranes are much less pronounced when compared to those seen in the distal tubule epithelium. Cells of the collecting tubule send cell membrane projections into the lateral intercellular space, while the basal cell membrane appears almost smooth (Fig. 14A). In the ducts, the intercellular spaces are often more prominent (not shown).

Wolffian Duct (Figs. 4, 6, 15)

The Wolffian duct is surrounded by dense connective tissue (Figs. 4, 6A,F, 15A–C). In the most frontal part of kidney, corresponding to the frontal segmental divisions, the epithelium of the duct resembles that of the collecting tubules, and is composed of cuboidal principal and intercalated cells (Figs. 6B, 15A). Both principal and intercalated cells are structurally similar to the cells of the collecting duct system; their apical surface, however, bulges into the lumen of the duct. In the more caudal regions, principal and intercalated cells become columnar and basal cells, restricted to the basal region of the epithelium, appear (Fig. 15B,C). In this region of the duct numerous secretory granules are found in the apical cytoplasm of principal cells (Fig. 15D). Lateral intercellular spaces are more dilated in the caudal regions of the duct and in these regions projections of the basal cell membrane reoccur (Fig. 14).

Peritoneal Funnel and Ciliated Tubule (Figs. 3, 5, 6, 16)

The epithelium of the peritoneal funnels and the ciliated tubules consists of ciliated cells with the conventional 9 + 2 microtubular arrangement and basally to centrally located nuclei (Fig. 16). A few minute microvilli cover the apical surface of the cells (Fig. 16A,C). As for the neck and intermediate segments, microtubules and densely packed filaments are found in the apical cytoplasm along with basal structures of the cilia, such as basal bodies, striated rootlets, and a terminal web (Fig. 16). The cytoplasm of these ciliated cells also contains a large number of mitochondria, a Golgi complex, endoplasmic reticulum, polyribosomes, lysosomes, and vesicles of different size and electron density (Fig. 16). The lateral cell membranes of the funnel cells form simple invaginations, whereas the basal cell membranes have no infoldings (Fig. 16B,C). In the ciliated tubule, the lateral cell membranes are without infoldings (Fig. 16A).

The cells are columnar and have a height of 11–13 μm in the funnels (Fig. 16C), but become cuboidal and decrease in height to 6-8 μm in the tubules (Fig. 16A). Peritoneal funnels connect with ventral nephrons via ciliated tubules (Figs. 3, 5). The external diameter of the tubules decrease gradually from the funnel opening toward the merger with the neck segment (Fig. 6B). In medial nephrons the ciliated tubules either end blindly and have no lumen or they open into internal ducts with a lumen only in the frontal segmental part of the kidney (Fig. 3). The ciliated tubules of medial nephrons are very narrow at their distal ends having outer diameters of only a few micrometers and a thin and plate-like epithelium.

DISCUSSION

The mesonephric kidneys of *Geotrypetes seraphini* are long and slender organs, broadest caudally and tapering toward the front. In its frontal part the kidney is subdivided into smaller segmental divisions (Fig. 2), a feature that has also been reported from other caecilians, e.g., *Hypogeophis rostratus* and *Siphonops annulatus* (see Brauer, 1902; Carvalho and Junqueira, 1999). Two nephron types are present in the mesonephros of *G. seraphini*. *Ventral nephrons* are connected to the coelom via a ciliated peritoneal funnel, while *medial nephrons* lack this connection. Ventral nephrons are identical to the primary and secondary nephrons ("Urnierenabschnitte") described from the studies on kidney development in *H. rostratus* by Brauer (1902). Thus, the ventral nephrons of *G. seraphini* may represent the first and second generation of nephrons, while medial nephrons reflect nephron generations appearing later in development. The presence of two nephron types, one connecting to a peritoneal funnel and the other with a ciliated tubule ending blindly in the loose connective tissue of the kidney is also reported for the aquatic *Typhlonectes compressicauda* (see Sakai et al., 1986), and is likely characteristic of at least the "higher" caecilians.

The mesonephric nephron in the amphibian species studied so far is composed of a Malpighian corpuscle and a renal tubule, which can be divided into six distinct sections: neck segment, proximal tubule, intermediate segment, early distal tubule, late distal tubule, and finally the collecting tubule, which opens into collecting ducts that lead the urine to the Wolffian duct (summarized in Møbjerg et al., 1998). Variation among amphibians in mesonephric nephron design includes differences in renal corpuscle size, nephron length, proximal and distal tubule segmentation, relative lengths of tubule segments, and the occurrence of ciliated tubules and their connections to peritoneal funnels.

In *Geotrypetes seraphini* the maximal dimensions of Malpighian corpuscles are 180 × 100 μm (present study). We found variation in the size of the Malpighian corpuscle between different regions of the same kidney and between kidneys of specimens kept either terrestrially or aquatically. The observed dimensional differences reflect differences in glomerular size as well as in the size of the urinary space as revealed by the size differences in the capsules of

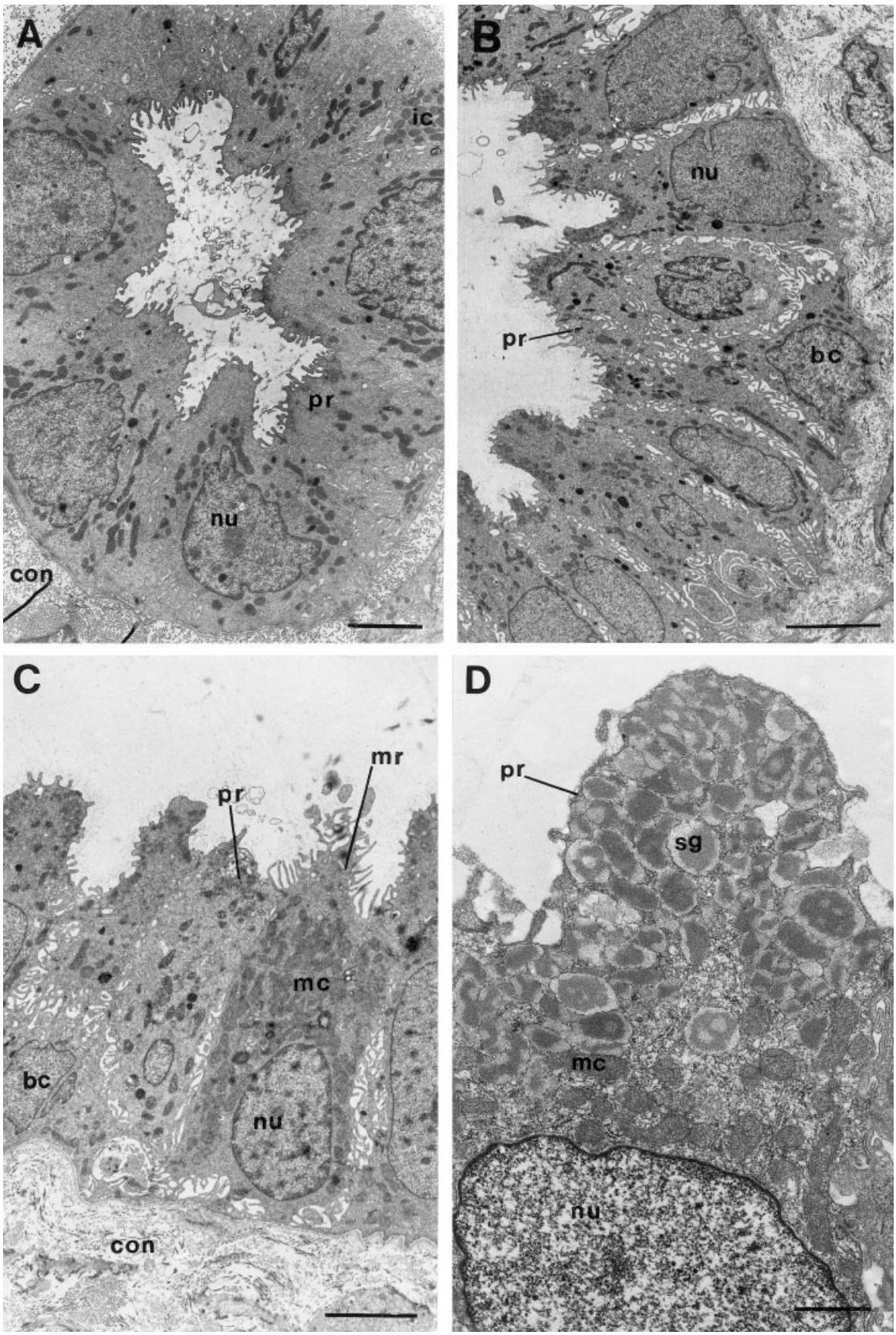


Fig. 15. *Geotrypetes seraphini*. Wolffian duct. TEM. **A:** Transverse section of the duct from a frontal segmental division of the kidney. **B,C,D:** From the more caudal part. Mitochondria-rich cells (mr) are found between principal cells (pr). Secretory granules (sg) are numerous in the apical cytoplasm of cells from the caudal part of the duct (**D**). bc, basal cells; con, connective tissue; mc, mitochondria, nu, nuclei. Scale bars = 3 μm (**A**), 5 μm (**B**), 4 μm (**C**), and 1 μm (**D**).

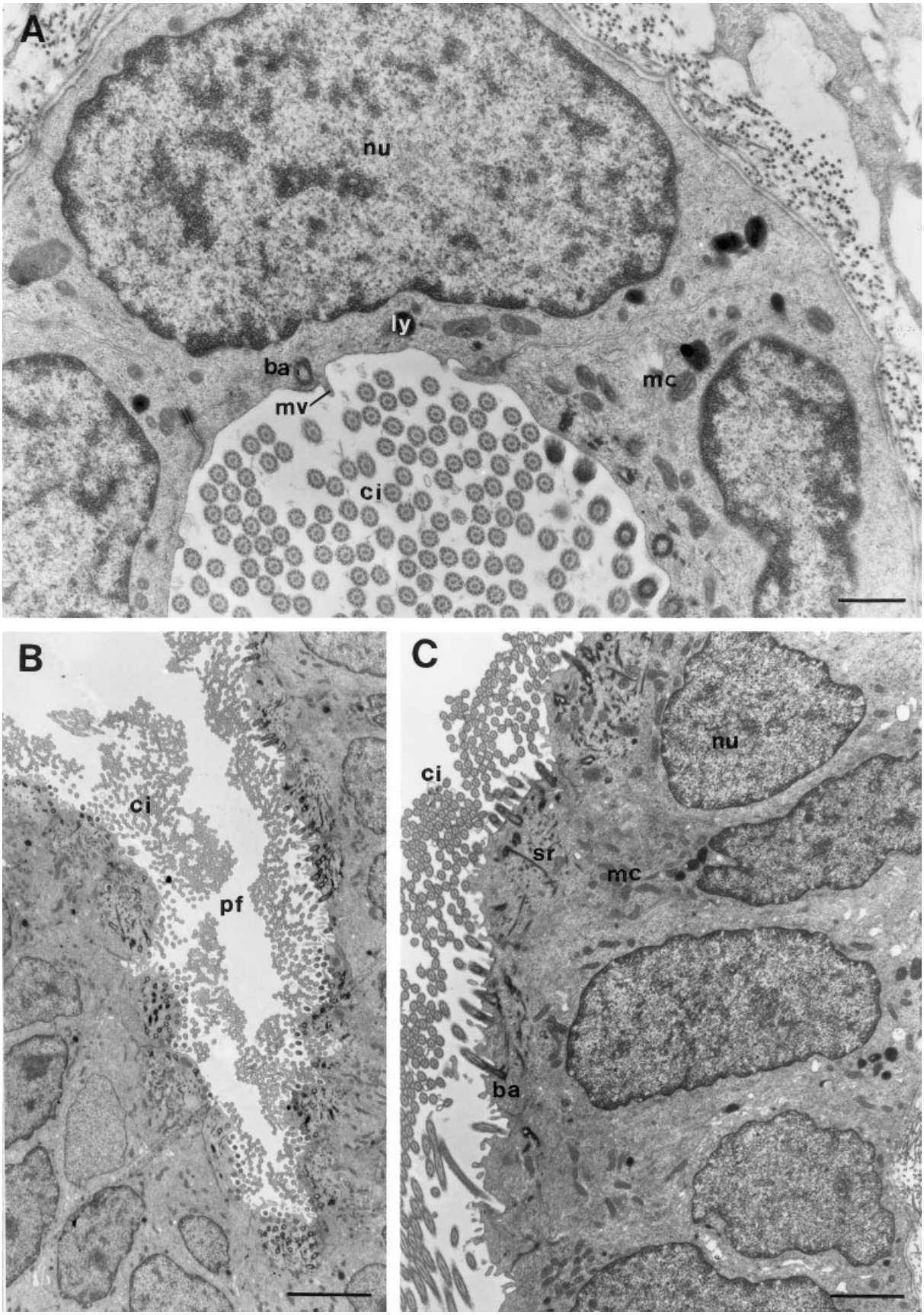


Fig. 16. *Geotrypetes seraphini.* TEM. **A:** Transverse section of the ciliated tubule. **B** and **C:** Longitudinal sections of the peritoneal funnel (pf). ba, basal body; ci, cilia; ly, lysosome; mc, mitochondria; mv, microvillus; nu, nuclei; sr, striated rootlets. Scale bars = 1 μm (**A**), 5 μm (**B**), and 2 μm (**C**).

Bowman. The filtration unit is small in *G. seraphini* when compared to that of other caecilians (Wake, 1970). Exceptionally large Malpighian corpuscles, with maximal dimension of up to 270 × 370 μm, are reported from the freshwater inhabitant *Typhlonectes compressicauda* (see Wake, 1970; Sakai et al., 1986).

As is the case for proximal tubules in other vertebrates, the cells of the amphibian proximal tubule are specialized for the uptake of an isotonic absorbate, which on the ultrastructural level involves the development of a luminal brush border, apical endocytotic apparatus, and lysosomal system as well as conspicuous lateral intercellular spaces and a basal labyrinth (Maunsbach, 1973; Maunsbach and Boulpaep, 1984; Sakai et al., 1988a; Fenoglio et al., 1996; Farias et al., 1998; Møbjerg et al., 1998). An intercalated cell type, the so-called bald-headed cell, is present in the proximal tubules of *Typhlonectes compressicauda* and *Geotrypetes seraphini* (see Sakai et al., 1988a; present study). Bald-headed cells have also been described from the salamander *Necturus maculosus* (summarized in Maunsbach and Boulpaep, 1984). Cells without a brush border are also found intercalated between the principal cells in the proximal tubule segment I of many teleost fish (reviewed in Hentschel and Elger, 1989). Hence, the absence of these cells in the proximal tubule of anurans may represent an apomorphy. A total of three morphologically different proximal tubule segments can be recognized in the *Geotrypetes* kidney. In the frontal segmental divisions of the kidney, the proximal tubule principal cells are variable in size and shape and the tubule has only a moderately developed brush border. Proximal tubules with similar cytology are not found in the mid-frontal to caudal part of the kidney. In these more caudal parts the cuboidal to columnar proximal tubule principal cells have a well-developed brush border and can be subdivided into two segments based primarily on cell height and density of the cytoplasm. Similar proximal tubule subdivisions have not been described for other caecilians (Welsch and Storch, 1973; Sakai et al., 1988a; Carvalho and Junqueira, 1999). At this stage it is difficult to conclude whether subdivisions are missing in other caecilians or just have not been recognized. In both salamanders and frogs the proximal tubule may, however, be subdivided in two segments based primarily on cellular and brush border height, as well as on the extent of the basal labyrinth (Maunsbach, 1973; Pons et al., 1982; Taugner et al., 1982; Uchiyama et al., 1990; Fenoglio et al., 1996). Clothier et al. (1978) subdivide the proximal tubule of the urodele *Amphiuma means* into three regions, based on the position of the nucleus and on the nature of the cytoplasmic inclusions. The authors do not report any differences between frontal and more caudal regions of the kidney. In some species of both frogs and salamanders the proximal tubule cannot be subdivided into morphological different regions, although cell size is often smaller towards the intermediate segment (Sakai and Kawahara 1983; Farias et al., 1998; Møbjerg et al., 1998). The variations in proximal tubule structure may reflect phylogenetic as well as functional differences. The significance of these variations is an obvious subject for further studies.

The amphibian distal tubule consists of two distal tubule segments, the early distal tubule and the late distal tubule. The latter opens into the first part of the collecting duct system, the collecting tubule. The early distal tubule is composed of a single cell type characterized by a large number of apical junctional complexes and a well-developed basal labyrinth, which together with a palisade arrangement of the large number of mitochondria often gives the cell a striated appearance (Stoner, 1977; Hinton et al., 1982; Taugner et al., 1982; Stanton et al., 1984; Sakai et al., 1986; Uchiyama et al., 1990; Fenoglio et al., 1996; Møbjerg et al., 1998; Carvalho and Junqueira, 1999; present study). In amphibians, urine concentration is hypo-osmotic to plasma and amphibians produce very dilute urine (see, e.g., Wiederholt and Hansen, 1980) in freshwater. The early distal tubule is the amphibian diluting segment and a significant amount of salt reabsorption occurs here (Stoner, 1977; Guggino et al., 1988; Dietl and Stanton, 1993). The amphibian late distal tubule is heterogeneous and considerable confusion exists on its nomenclature. Up to three morphologically different sections, Sections I–III, may be present (Møbjerg et al., 1998). The late distal tubule section I is distinctly defined, the cells constituting this section having a large nucleus and well-developed lateral and basal labyrinths. The late distal tubule sections II and III represent the gradual transition between section I and the distinctly defined collecting tubule. Collecting tubules and ducts are heterocellular, composed of principal and intercalated mitochondria-rich cells (summarized in Møbjerg et al., 1998). As may be perceived from the above description, the amphibian mesonephric distal nephron is a complex structure with several subdivisions that each have their own structural characteristics. The presence of several different segments within the distal tubule has resulted in an inconsistent nomenclature of the tubular segments. The organization and nomenclature of the amphibian distal nephron was discussed in detail by Møbjerg et al. (1998). In *Geotrypetes seraphini* the distal tubule is subdivided into an early distal tubule and a late distal tubule. Distal tubule subdivision is only clearly recognizable at the electron microscopic level. The late distal tubule is not further subdivided and the entire tubule corresponds to section I. Osmiophilious intercalated cells were found interposed between principal cells of this section in the mesonephros of the toad *Bufo bufo* and similar cells are observed in the present study of *G. seraphini* (see Møbjerg et al., 1998; present study). These cells are

morphologically different from the intercalated cells of the collecting tubules and ducts. In *G. seraphini* the late tubule section I opens directly into the collecting tubule. The distal nephron in *Typhlonectes compressicauda* can be divided into an early distal tubule (distal tubule) and a tubule segment corresponding to section III of the late distal tubule (connecting tubule) (Sakai et al., 1986, 1988b). Based on their light microscopic observations, Carvalho and Junqueira (1999) could not subdivide the distal tubule of *Siphonops annulatus*. The distal tubule segment reported from their study corresponds to the early distal tubule. In summary, it seems that an early distal tubule and a heterocellular collecting duct system composed of collecting tubules and ducts are present in all amphibians. A large variation is, however, seen in the presence of late distal tubule segments, a variation that probably reflects phylogenetic as well as functional differences.

The original vertebrate kidney, archinephros or holonephros, is believed to have covered the entire length of the coelom, consisting of paired segmental tubules with associated glomeruli, archinephrons (Price, 1897; Goodrich, 1958). Each tubule opened into the coelom through a ciliated nephrostome and was connected to the archinephric duct, which led to the exterior (Goodrich, 1958). Nephrons were present at some time in all body segments, but later were restricted to the trunk region of the animal. A tendency for the most anterior tubules to develop and function in early life, and for the more posterior tubules to develop and function later in life led to the differentiation into pro-, meso-, and metanephros (Goodrich, 1958).

The kidney in embryos of the caecilian *Hypogeophis rostratus* was described by Brauer (1902) and it resembled to a high degree the hypothetical holonephros, although the pronephros and mesonephros were separated by a small zone of segments without functional nephrons. According to Brauer (1902) the kidney in ancestral caecilians was present in all the segments of the trunk, and it was therefore essentially a holonephros. Wrobel and Süβ (2000), in a recent account on the kidney of *Ichthyophis kohtaoensis*, studied the development of the urogenital organs in larvae. In *I. kohtaoensis*, embryos possess a well-developed, large pronephros beginning immediately behind the branchial region and extending over ~10 body segments. Interestingly, the kidney of larval *I. kohtaoensis* does not resemble a holonephros: the caudal end of the pronephros overlaps with the mesonephros. Such an overlapping of the two kidney forms was also observed by Semon (1891) in *Ichthyophis glutinosus*. Wrobel and Süβ (2000) compare their findings in *I. kohtaoensis* with results obtained from bovine embryos and argue that the vertebrate gonad is a modified homolog of the pronephros, and therefore does not develop from mesenchymal cells, the coelomic mesothelium, or elements of the mesonephros as previously proposed. The authors argue that nephrostomial tubules of pronephric origin are the immediate precursors of the blastemas for adrenocortical, rete, gonadal, and Müllerian infundibular development.

On the basis of ontogenetic studies, the vertebrate nephron can be divided into two major parts, the proximal nephron and the distal nephron (Hentschel and Elger, 1989). In the mesonephros of amphibians the proximal nephron comprises the Malpighian corpuscle, a ciliated neck segment, the proximal tubule, and a ciliated intermediate segment (Fig. 3). The distal nephron is constituted by the early and late distal tubules (Fig. 3). The latter open into the collecting tubule, which is the first unbranched portion of the collecting duct system. This nephron type is also present in the mesonephros of archaic bony fish, such as polypterids, and in Dipnoi, and it may therefore be representative of the vertebrate archinephron (Hentschel and Elger, 1989). Far from all vertebrates comply with this nephron type. Within different vertebrate groups there is great variation in nephron structure. The extremes are represented by the aglomerular nephrons of highly advanced marine teleosts, which have lost the glomeruli, and by the atubular nephrons of hagfish (Myxinidae), in which the exceptionally large glomeruli are connected directly to the nephric duct via short vestigial tubules (Hickman and Trump, 1969; Hentschel and Elger, 1987; Fels et al., 1989; Hentschel, 1997). It is a matter of debate whether the atubular nephron of hagfish is a plesiomorphic condition representing the vertebrate archinephron or whether it represents an autapomorphy resulting from regressive evolution of a more elaborate nephron type in early vertebrates.

Smith (1953) believed that the glomerulus was unique to vertebrates and developed as a device to excrete water during the invasion of the freshwater environment. Modern comparative studies, however, suggest that this design is by no means restricted to vertebrates, but is widespread among invertebrates in the form of metanephridial systems (Ruppert and Smith, 1988; Bartolomaeus and Ax, 1992). Therefore, vertebrates probably inherited the components of the archinephron from their invertebrate ancestors (Ruppert, 1994). In metanephridial systems ultrafiltration occurs from a blood vessel through a filtration barrier and into the coelomic cavity (Fig. 17A). From the coelom the filtrate enters the metanephridium by way of a ciliated funnel. The structural and functional unit of the vertebrate kidney, the nephron, is a modified metanephridial system, the glomerulus being the site of ultrafiltration (Ruppert, 1994).

In the amphibian kidney systems we find a series of different associations between the nephron and ciliated tubules opening into the coelom via peritoneal funnels. These systems may therefore be ideal for the study of vertebrate nephron evolution. Figure 17B–D illustrates the series of evolutionary

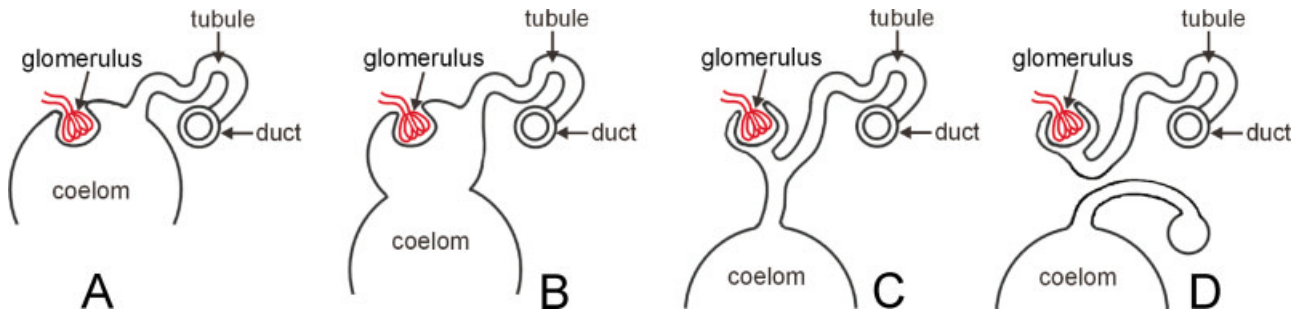


Fig. 17. Evolutionary events that may have led to the development of the Malpighian corpuscle of the vertebrate nephron. **A:** An external glomerulus is situated in the coelom. The proximal end of the renal tubule opens into the coelom; the distal end connects with the nephric duct. **B:** A small coelomic chamber has formed around the external glomerulus. This is the situation in the pronephros of amphibian larvae. **C:** The coelomic chamber becomes the capsule of Bowman, which surrounds the glomerulus, and a Malpighian corpuscle is formed. The renal tubule connects to the coelom via ciliated peritoneal funnels. This is the situation in the mesonephros of adult salamanders and caecilians. **D:** The renal tubule loses its connection to the coelom and in anuran amphibians the peritoneal funnels open into the peritubular blood vessels.

events that might have led from a typical metanephric system such as the pronephros to the formation of a nephron with an internal glomerulus, typical of the mesonephros and metanephros.

In the amphibian pronephros, the nephron is composed of two principal parts—the pronephric filtration unit and the pronephric tubule. In frogs and salamanders the filtration unit is an external glomerulus connected to a single convoluted tubule, which opens into the coelom via nephrostomes (Fig. 17B; Fox, 1963; Christensen, 1964; Viertel and Richter, 1999; Møbjerg et al., 2000). In the caecilian *Ichthyophis kohtaoensis* the glomeruli form a single large glomus (Wrobel and Süβ, 2000). The space between the glomeruli may communicate with the coelomic cavity and the glomeruli can therefore be viewed as intermediate between external and internal (Wrobel and Süβ, 2000). This would indicate that the caecilian glomus represents a derived condition.

The Malpighian corpuscle of the amphibian mesonephric nephron consists of an internal glomerulus and a surrounding epithelial capsule, the capsule of Bowman. Two ciliated segments are present in the amphibian mesonephric nephron, the neck segment, and the intermediate segment. A common characteristic of nephridia is the presence of ciliated cells; the ciliated segments of the vertebrate nephron are therefore of special interest when comparing this structure with the nephridia of invertebrates. Little is known of the function of vertebrate ciliated segments, the general idea being that they are involved in fluid propulsion (Hentschel and Elger, 1989). In caecilians and salamanders this may involve taking up fluid from the body cavity through peritoneal funnels, which open into the neck segment (Fig. 17C; Clothier et al., 1978; Sakai and Kawahara, 1983; Sakai et al., 1986, 1988a; Carvalho and Junqueira, 1999; present study). However, in *Geotypetes seraphini* the ciliated tubules are very narrow at their distal ends and therefore probably contribute little if any fluid uptake from the body cavity. Sakai et al. (1986) do not report any difference in diameter along the course of the ciliated tubule, and the situation may therefore be different in the aquatic *Typhlonectes compressicauda*. In anurans, the nephron has lost its connection to the coelom and the peritoneal funnels open into the peritubular vessels surrounding the nephrons (Fig. 17D; Morris, 1981; Uchiyama et al., 1990; Møbjerg et al., 1998).

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