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Observations on Variation in the Ultrastructure of the Proximal Testicular Ducts of the Ground Skink, *Scincella lateralis* (Reptilia: Squamata)

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ABSTRACT The North American Ground Skink, *Scincella lateralis*, is a member of the most speciose family of lizards, the Scincidae. The only descriptions of the testicular ducts of skinks concern the light microscopy of 13 species in eight other genera. We combine histological observations with results from transmission electron microscopy on a sample of skinks collected throughout the active season. The single rete testis has squamous epithelium with a large, indented nucleus and no junctional complexes between cells or conspicuous organelles. Nuclei of sperm in the rete testis area are associated with cytoplasmic bodies that are lost in the ductuli efferentes. The ductuli efferentes have both ciliated and nonciliated cells and show little seasonal variation except for the narrowing of intercellular canaliculi when sperm are absent. When the ductus epididymis contains sperm, the anterior one-third lacks copious secretory material around luminal sperm, whereas in the posterior two-thirds sperm are embedded in a dense matrix of secretory material. Light and dark principal cells exist and both contain saccular, often distended rough endoplasmic reticula, and widened intercellular canaliculi that bridge intracellular spaces. Junctional complexes are lacking between principal cells except for apical tight junctions. Electron-dense secretory granules coalesce at the luminal border for apocrine release. The cranial end of the ductus deferens is similar in cytology to the posterior ductus epididymis. Each of the nine squamates in which the proximal testicular ducts have been studied with electron microscopy has some unique characters, but no synapomorphies for squamates as a group are recognized. J. Morphol. 274:429–446, 2013.

KEY WORDS: testicular ducts; skinks; squamata; ultrastructure

INTRODUCTION

The lizards and snakes, order Squamata, are the largest group of living amniotes with some 9,193 known species (Uetz, 2012). The largest group of lizards is the cosmopolitan family Scincidae that includes 1,516 species commonly known as skinks. In this article, we present the first ultrastructural observations on the anterior testicular ducts of a skink, the Ground Skink, *Scincella lateralis*, which is commonly found in the southeastern United States. Other species in the genus are known from Mexico and Asia (Honda et al., 2003; Garcia-Vázquez et al., 2010).

The only previous studies on skinks involve histology of testicular ducts of *Eumeces* (= *Plestiodon*) *fasciatus* from the eastern United States (Reynolds, 1943), *Chalcides ocellatus* and *Scincus scincus* from northern Africa (Badir, 1958), and nine additional species from Africa, Asia, Australia, and New Zealand (Dufaure and Saint Girons, 1984). Ultrastructural descriptions of the testicular ducts of lizards are limited to *Calotes versicolor* (Meeran et al., 2001) and *Sitana ponticeriana* (Akbarsha et al., 2006a,b, 2007) of the Agamidae, *Lacerta* (= *Zootoca) vivipara* (Mesure et al., 1991) and *Podarcis sicula* (Desantis et al., 2002) of the Lacertidae, *Tropidurus itambere* (Ferreira et al., 2009) of the Tropiduridae, and *Hemidactylus turcicus* (Rheubert et al., 2010) of the Gekkonidae. For snakes, ultrastructure of the testicular ducts has been described for *Seminatrix pygaea* of the Natricidae (Sever, 2004, 2010), *Agkistrodon piscivorus* of the Viperidae (Siegel et al., 2009; Trauth and Sever, 2011), *Pantherophis obsoletus* of the Colubridae (Trauth and Sever, 2011), and *Pelamis platurus* of the Elapidae (Sever and Freeborn, 2012).

Much variation has been noted in the ultrastructure of the ductus epididymis of squamates studied previously. We hypothesize that *S. lateralis* will show significant variation from other squamates in

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some epididymal characters, but conservation of other characters will help establish ancestral states for the evolution of testicular ducts in squamates.

MATERIALS AND METHODS

Twelve mature ground skinks *S. lateralis* Say, 1823, from 33 to 42 mm snout-vent length (SVL) were examined after preparation for light microscopy (LM) and four of these individuals were also prepared for transmission electron microscopy (TEM). Specimens were collected in the vicinity of Hammond, Tangipahoa Parish, Louisiana on the following dates, with numbers collected in parentheses: March 12, 2006 (1), April 7, 2006 (1), April 9, 2008 (1), May 12, 2012 (1), June 6, 2009 (1), June 10, 2006 (2), September 27, 2011 (1), September 29, 2011 (1), October 27, 2006 (2), and November 5, 2009 (1). Specimens were sacrificed within 24 hr of capture, and single individuals collected April 9, 2008, June 6, 2009, September 27, 2011, and October 27, 2006 were selected for TEM. Note that ground skinks that occur in this region become inactive and difficult to collect during the colder months (December–February) and warmest months (July and August).

Specimens were euthanized by lethal interperitoneal injection (3–5 ml) of 10% sodium pentobarbital in 70% ethanol. This procedure was approved by the Institutional Animal Care and Use Committee of Southeastern Louisiana University, Hammond, Louisiana. After death, SVL was measured from the tip of the snout to the posterior end of the cloacal orifice. Total length was also measured and recorded. The left reproductive tract, consisting of the testis, kidney, and efferent ducts, was removed from each specimen and fixed in 10% neutral buffered formalin (NBF) for LM. For four specimens mentioned previously, right reproductive tracts were placed in Trump's fixative (2.5% glutaraldehyde and 2.5% formaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 (Electron Microscopy Sciences, Hatfield, PA, USA) for electron microscopy.

Tissues previously fixed in NBF were rinsed in deionized water, dehydrated through a series of ethanol (70, 80, 95, and 100%), cleared in two changes of toluene, and placed in melted paraffin under vacuum for a period of 24 hr. Tissues were then embedded in paraffin blocks that were allowed to harden before 10 μm thick sections were cut with a MR3 rotary microtome (RMC Instruments, Tucson, AZ, USA) and affixed to albumenized slides. Alternate slides from each specimen were stained with hematoxylin-eosin (general histology), bromphenol blue (BB, for proteins), and treated with the periodic acid-Schiff procedure (PAS, for neutral carbohydrates) counterstained with Alcian blue 8GX at pH 2.5 (AB, for carboxylated glycosaminoglycans). Procedures followed Hayat (1993). A Leica DM2000 compound microscope was used for viewing slides, and photographs were taken with a Leica DF420 attached digital camera (Leica Microsystems, Wetzlar, Germany).

Tissues for TEM were rinsed in deionized water, post fixed in 2% osmium tetroxide, dehydrated through a graded series of ethanol (same as LM), cleared in propylene oxide, and embedded in epoxy resin (Embed 812, Electron Microscopy Sciences, Hatfield, PA). Plastic sections were cut using a RMC MT7 ultramicrotome and DiATOME (Biel, Switzerland) diamond knives at 1 μm and 70 nm. Toluidine blue was used to stain 1 μm thick sections. Sections 70 nm in thickness were placed on uncoated 200 mesh copper grids (Electron Microscopy Sciences, Hatfield, PA) and stained with uranyl acetate and lead citrate. Grids were viewed using a JEOL 100 transmission electron microscope (JEOL Ltd., Tokyo, Japan) and photographed using a L3C CCD digital camera (Scientific Instruments and Applications, Duluth, GA). Illustrations of both light and electron micrographs were made with Adobe Photoshop 7.0 (Adobe Systems, San Francisco, CA, USA).

RESULTS

Spermiogenesis and spermiation were observed in specimens examined from March to June. We were unable to collect skinks in July and August, possibly because the high temperatures limit surface activity. Skinks from September have inactive testes, but recrudescence begins in October. Mating has been observed in the areas where samples were collected in March (Fig. 1A). Consistent with many other lizards (Rheubert et al., 2010), only one rete testis tubule extends intratesticularly from the seminiferous tubules into the extratesticular sheath containing the ductuli efferentes and the ductus epididymis. Thus, the rete testis has an intratesticular and extratesticular portion (Fig. 1B,C). The ductus epididymis occurs along the caudal three-fifths of the testis, and the rete testis enters the sheath where ductuli efferentes are numerous and anterior to the appearance of the ductus epididymis. Ductuli efferentes also occur along the anterior portion of the epididymis and are the only tubules in the duct system that are ciliated. When filled with sperm, the ductus epididymis has a distinct anterior and posterior portion, as described below.

The rete testis did not show any reactions to PAS/AB or to BB in any specimens. The ductus epididymis generally has a weak reaction to PAS and no reaction to AB, but the ductuli efferentes has a moderate reaction to PAS (Fig. 2A,B, E). The ductus epididymis and ductuli efferentes both show a strong reaction with BB for proteins throughout the year, even when sperm are absent (Fig. 2C,D,F). The accuracy of staining was shown by simultaneously staining portions of the kidney in which the sexual segment was PAS+ and BB+ and the proximal convoluted tubules AB+ as reported by Sever and Hopkins (2005). Squamates examined in previous studies have all shown a reaction, especially in the ductus epididymis, to neutral carbohydrates and/or proteins, often stronger for one product than the other (Dufaure and Saint Girons, 1984; Sever and Freeborn, 2012).

April

In the specimen from April whose proximal testicular ducts were studied by TEM, spermatogenesis is actively occurring in the testis (Fig. 3A) and the ductus epididymis is filled with sperm (Fig. 3B). Sperm also occur in the rete testis and ductuli efferentes but are not as tightly packed as in the ductus epididymis. We were unable to find the rete testis in our semithin and ultrathin sections.

The ductuli efferentes consist of simple cuboidal epithelium with large, irregular nuclei that are relatively heterochromatic and possess prominent nucleoli (Fig. 3C). Ciliated cells are more numerous than nonciliated cells. Intercellular spaces are scattered throughout the cytoplasm and intercellular canaliculi are widened (Fig. 3C), although apically they form tight junctions (Fig. 3D). Organ-

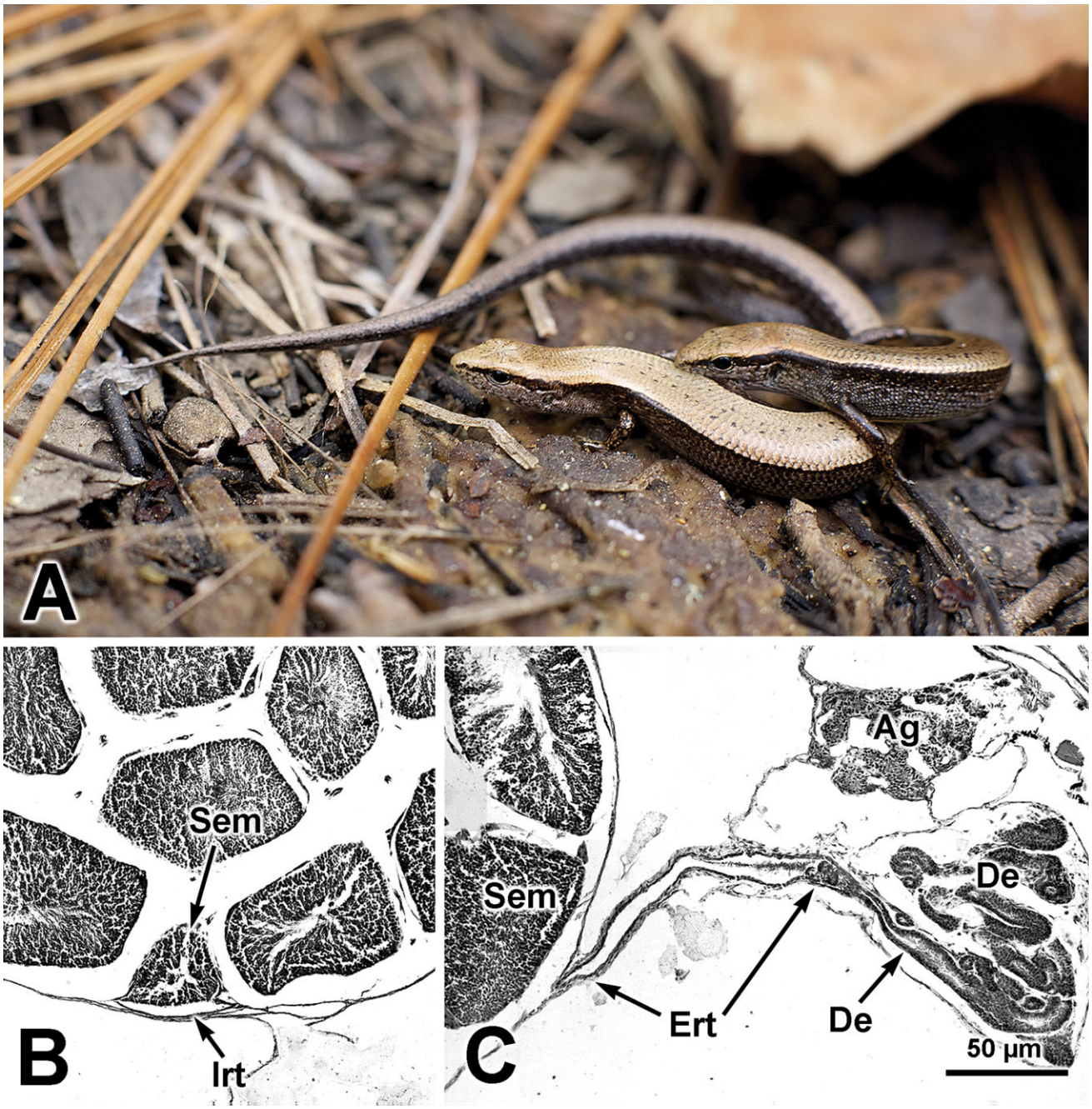

Fig. 1. *Scincella lateralis*. (**A**) Male (right) discovered while mating with a female on March 11, 2012, Tangipahoa Parish, Louisiana. Photo by D. S. Siegel. (**B, C**) Light micrographs of proximal testicular ducts of a 39.5 mm SVL male collected April 9, 2008 stained with hematoxylin and eosin. (B) Seminiferous tubules merging into an intratesticular rete testis. (C) Extratesticular rete testis merging into ductuli efferentes. Ag, adrenal gland; De, ductuli efferentes; Ert, extratesticular rete testis; Irt, intratesticular rete testis; Sem, seminiferous tubules. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

elles are not conspicuous except for small, dense mitochondria. Tiny coated vesicles are associated with the microvilli of nonciliated cells (Fig. 3D).

The anterior one-third of the ductus epididymis consists largely of tall and narrow principal cells, 20–30 μm in height, with basal nuclei (Fig. 4A). True "basal cells" characteristic of pseudostratified epithelium of the ductus epididymis of other vertebrates are not noticeable in this portion of the duct. The principal cells can be divided into the anterior light cells, which are more numerous, and anterior dark cells based solely on relative electron density (Fig. 4A). Sperm are randomly spread throughout a lumen that is largely devoid of a noticeable secretory product (Fig. 4B). Membrane bound, spherical, electron-dense secretory granules,

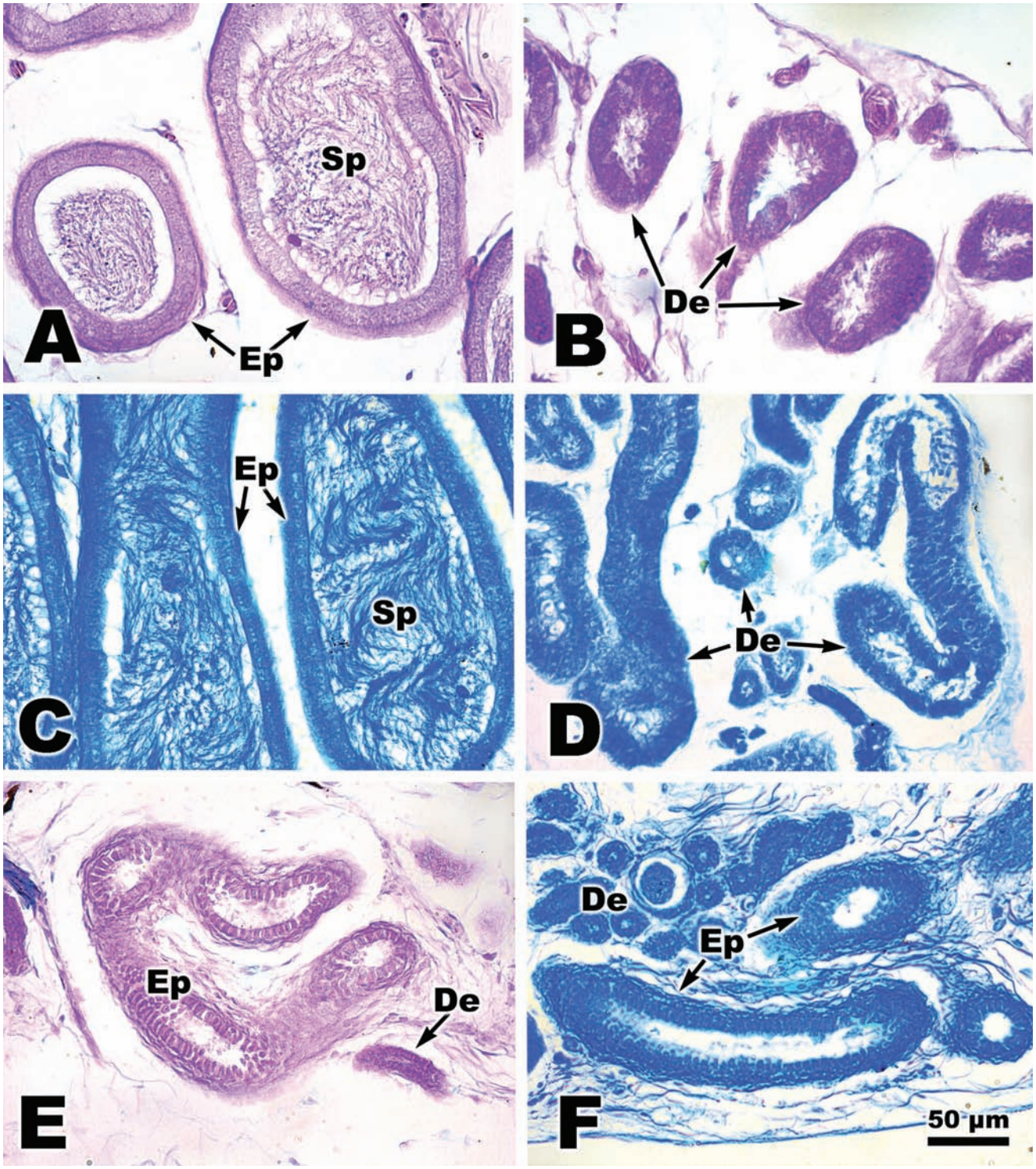

Fig. 2. *Scincella lateralis*. Histochemical reactions of paraffin sections of ductuli efferentes and ductus epididymis to periodic acid and Schiff's reagent (PAS) and Alcian blue for carbohydrates (**A**, **B**, **E**) and bromphenol blue (BB) for proteins (**C**, **D**, **F**). (A–D) Male 41.1 mm SVL collected June 6, 2009. (A) Weak reaction with PAS for the ductus epididymis. (B) Moderate reaction with PAS for the ductuli efferentes. (C) Strong reaction with BB for the ductus epididymis. (D) Strong reaction with BB for the ductuli efferentes. (E, F) Male 33.0 mm SVL collected September 27, 2011. (E) Weak reaction with PAS of the ductus epididymis and moderate reaction of the ductuli efferentes. (F) Strong reactions with BB of the ductuli efferentes and ductus epididymis. De, dutuli efferentes; Ep, ductus edpididymis; Sp, sperm.

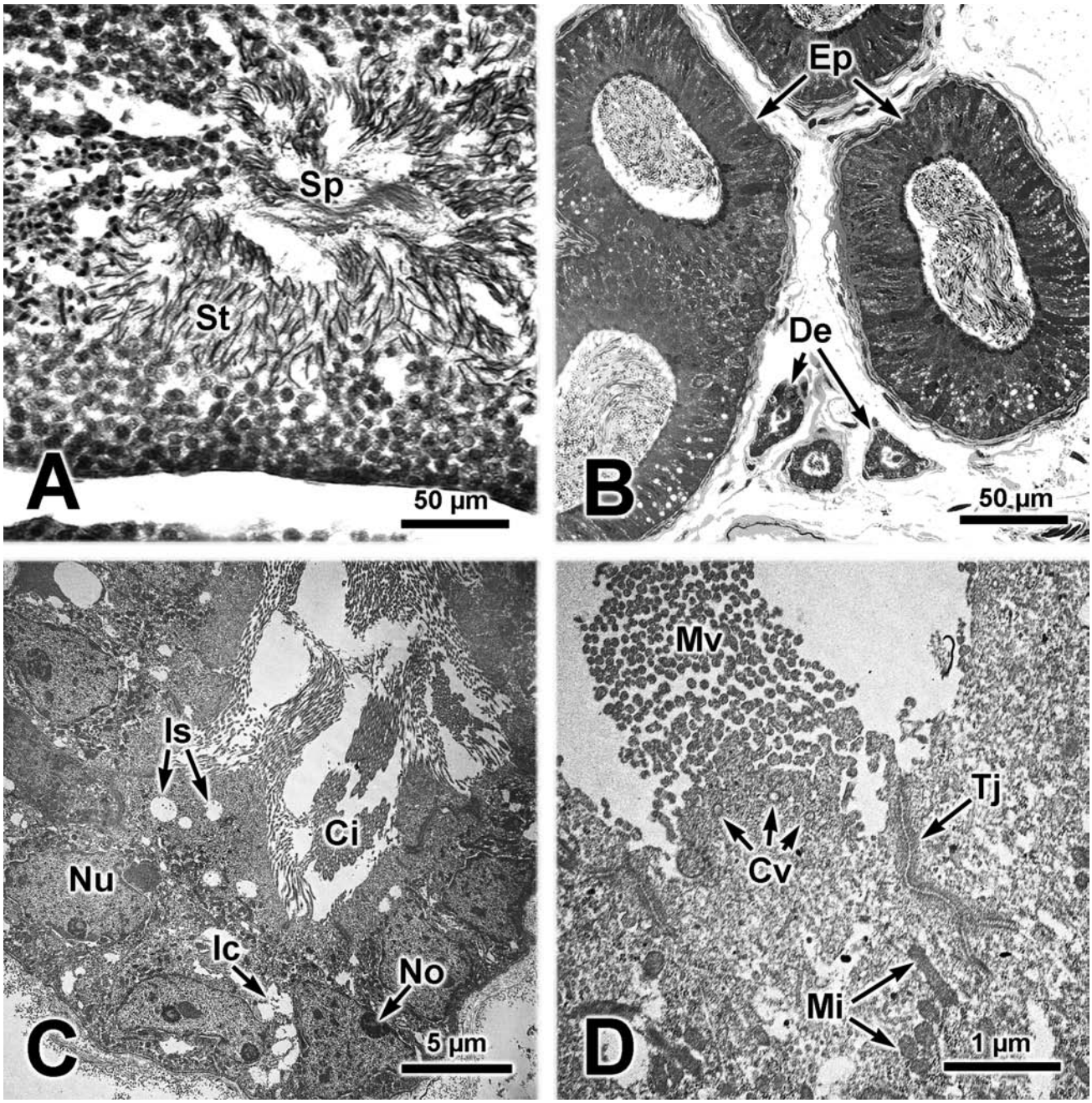

Fig. 3. Testis and proximal efferent ducts from a 39.5 mm SVL *Scincella lateralis* collected April 9, 2008. (**A**) Light micrograph (LM) of a plastic section of the testis stained with toluidine blue. (**B**) LM of a plastic section showing overview of anterior portion of the ductuli efferentes and ductus epididymis stained with toluidine blue. (**C**) TEM of the ductuli efferentes. (**D**) Apical cytoplasm of a non-ciliated cell of the ductuli efferentes. Ci, cilia; Cv, coated vesicles; De, ductuli efferentes; Ep, ductus epididymis; Ic, intercellular canaliculi; Is, intracellular space; Mi, mitochondria; Mv, microvilli; No, nucleolus; Nu, nuclei; Sp, sperm; St, spermatids; Tj, tight junction.

however, are numerous in the apical cytoplasm of both light and dark cells (Fig. 4C). Granules generally range from 0.25 to 0.50 µm. Tight junctions occur between cells at the luminal border (Fig. 4C). Junctional complexes are absent elsewhere along the intercellular canaliculi. Throughout the principal cells, the cytoplasm is characterized by an abundance of rough endoplasmic reticulum (Rer) and numerous intracellular spaces. These intercellular spaces are not vacuoles because they appear to lack membranes. The intercellular canaliculi, especially in the basal half of the principal cells, have widened and narrow regions. The Rer in the basal half of the cells often consists of dilated cisternae and these

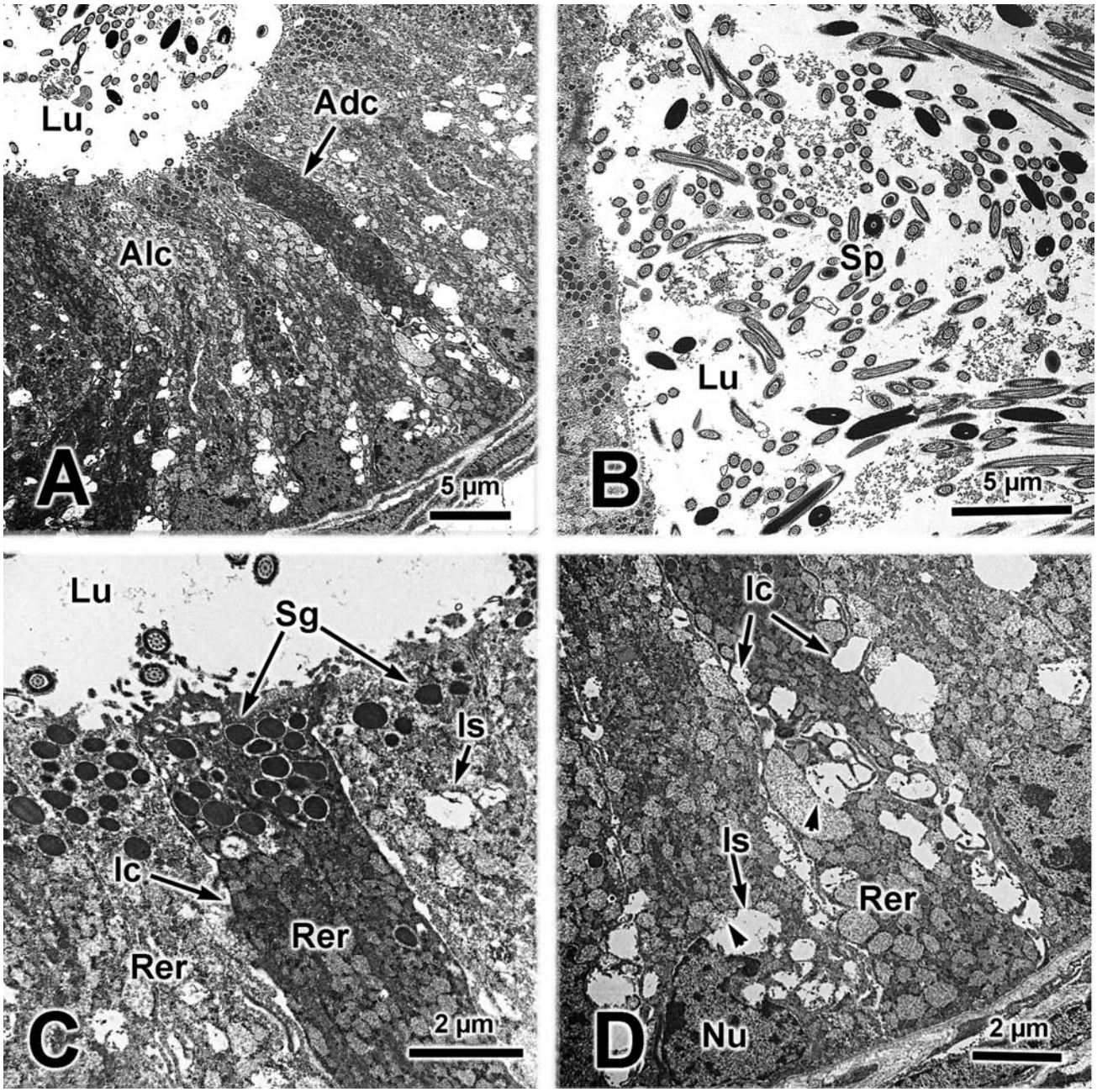

Fig. 4. *Scincella lateralis*, same specimen as used for Figure 3, illustrating TEMs of the anterior portion of the ductus epididymis. (**A**) Overview of a group of principal cells. (**B**) Sperm in the lumen. (**C**) Apical cytoplasm. (**D**) Basal cytoplasm. Unlabeled arrows indicate Rer abutting against intercellular canals and spaces. Adc, anterior dark cell; Alc, anterior light cell; Ic, intercellular canaliculi; Is, intracellular space; Lu, lumen; Nu, nucleus; Rer, rough endoplasmic reticulum; Sg, secretory granules; Sp, sperm.

abut on the intracellular spaces as well as the intercellular canaliculi (Fig. 4D, unlabeled arrows).

The posterior two-thirds of the ductus epididymis has even more intracellular spaces than the anterior one-third (Fig. 5A,B). Also, the sperm in the lumen are mixed in a thick matrix of granular secretory material (Fig. 5B,C). True squamous basal cells are found sporadically abutting the basal lamina (Fig. 5B). Two types of principal cells are apparent. In one type, called the posterior dark cells, Rer is densely abundant up to the luminal border where a thickened dense luminal border occurs (Fig. 5D). In the other type of cell, Rer do not occur at the luminal border, which is not as thick, and secretory granules are more common; these will be called posterior light cells (Fig. 5D).

In the posterior light cells, the density of the secretory granules closely matches the density of the secretion in the lumen (Fig. 6A). In posterior dark cells, the thickened luminal border is more electron

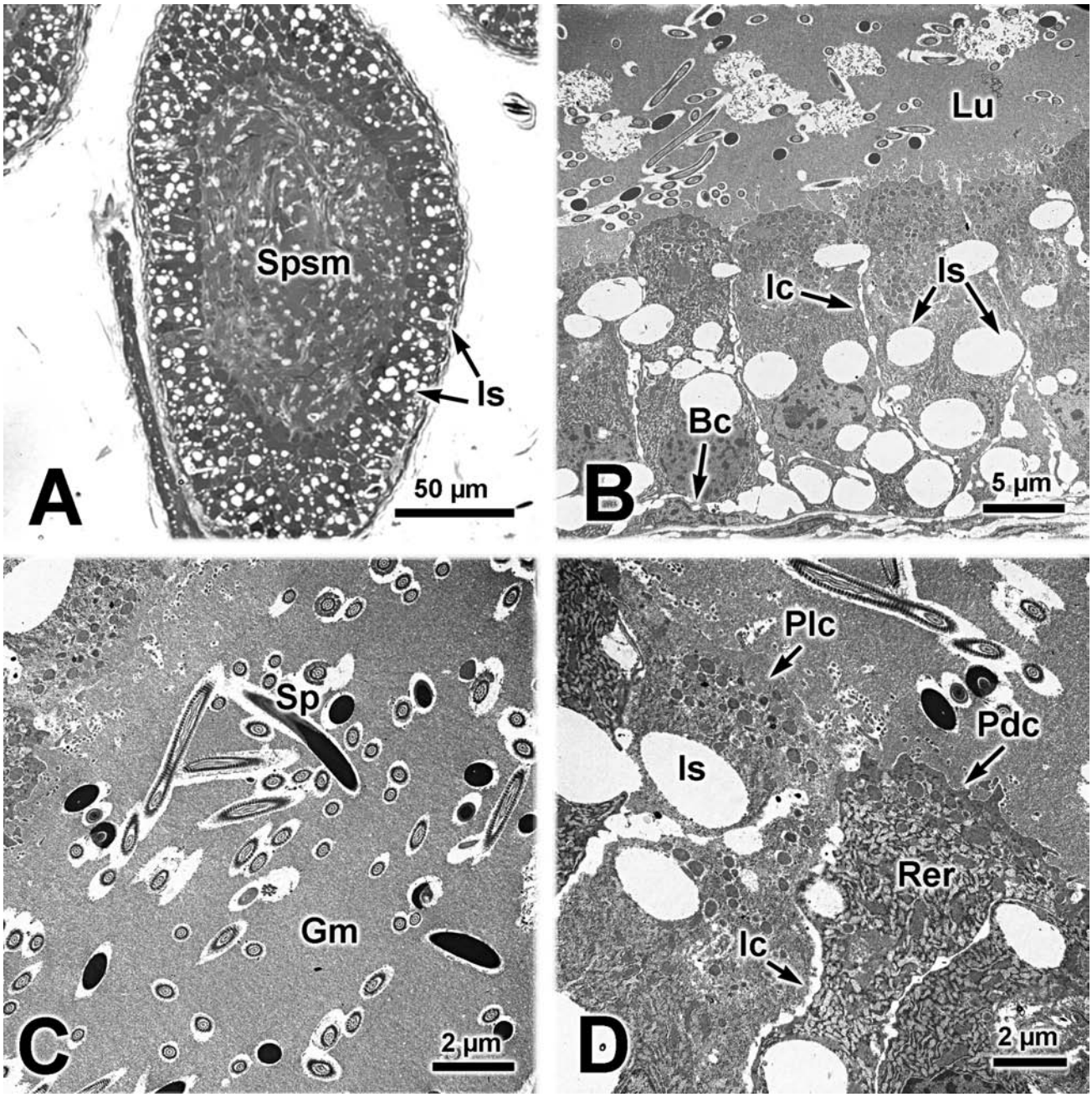

Fig. 5. *Scincella lateralis*, same specimen as used for Figures 3 and 4, illustrating the posterior portion of the ductus epididymis. (**A**) Light micrograph of a plastic section stained with toluidine blue. (**B**) TEM overview of a group of principal cells. (**C**) TEM of sperm in the lumen. (**D**) TEM of variation in the apical cytoplasm of principal cells. Bc, basal cell; Gm, granular material; Ic, intercellular canaliculi; Is, intracellular space; Lu, lumen; Pdc, posterior dark cell; Plc, posterior light cell; Rer, rough endoplasmic reticulum; Sp, sperm; Spsm, sperm in secretory matrix.

dense than the overall luminal secretion, and it appears that "droplets" from the border detach into the lumen (Fig. 6B). Tight junctions can be found apically in light cells (Fig. 6A) but the plasma membranes of adjacent dark cells meld into the thickened luminal border. The thickened luminal border of the dark cells seems to be a consequence of coalescing of secretory granules (Fig. 6B).

Basally, the cisternae of the Rer are not as distended as in the anterior ductus epididymis, but again the Rer makes direct membrane contact with widened areas of intercellular canaliculi and with intracellular spaces (unlabeled arrows, Fig. 6C). The widened spaces also often come in contact with nuclear membranes (Fig. 6C). The intercellular canaliculi lack junctional complexes basally

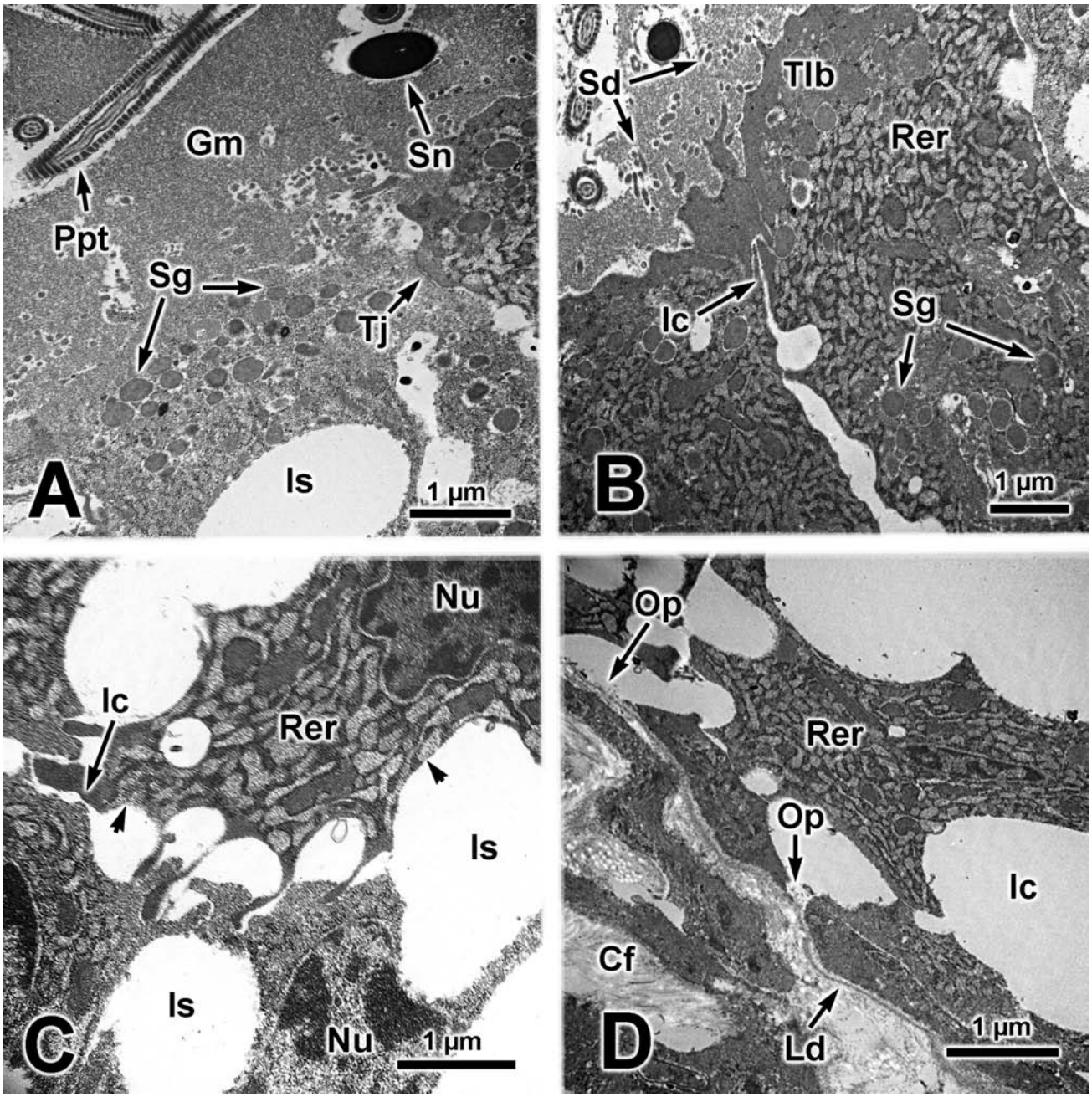

Fig. 6. *Scincella lateralis*, same specimen as used for Figures 3–5, showing more ultrastructural detail of the posterior portion of the ductus epididymis. (**A**) Apex of a posterior light cell that lacks extensive Rer and a thickened dense luminal border. (**B**) Luminal border of a posterior dark cell with dense aggregates of Rer and a thickened dense luminal border. (**C**) Perinuclear cytoplasm of several principal cells showing intercellular transport between cells. Unlabeled arrows indicate Rer abutting against intercellular canals and spaces; (**D**) Basal borders of several principal cells. Cf, collagen fibers; Gm, granular material; Ic, intercellular canaliculi; Is, intracellular space; Ld, lamina densa; Nu, nucleus; Op, opening of the intercellular canaliculi; Ppt, principal piece of the tail; Rer, rough endoplasmic reticulum; Sd, secretory droplets; Sg, secretory granules; Sn, sperm nucleus; Tj, tight junction; Tlb, thickened luminal border.

and have wide openings into the lamina lucida of the basement membrane (Fig. 6D).

June

In the June individual examined with TEM, the testis are still undergoing spermiogenesis (Fig. 7A), and sperm can be found throughout the rete testis, ductuli efferentes, and ductus epididymis. Thin sections through the rete testis were obtained, and the extreme squamous nature of the rete is apparent in a low-magnification TEM, with the height of cells generally 1.2–1.6 µm (Fig. 7B). Notice that nuclei of sperm in the lumen of the rete testis

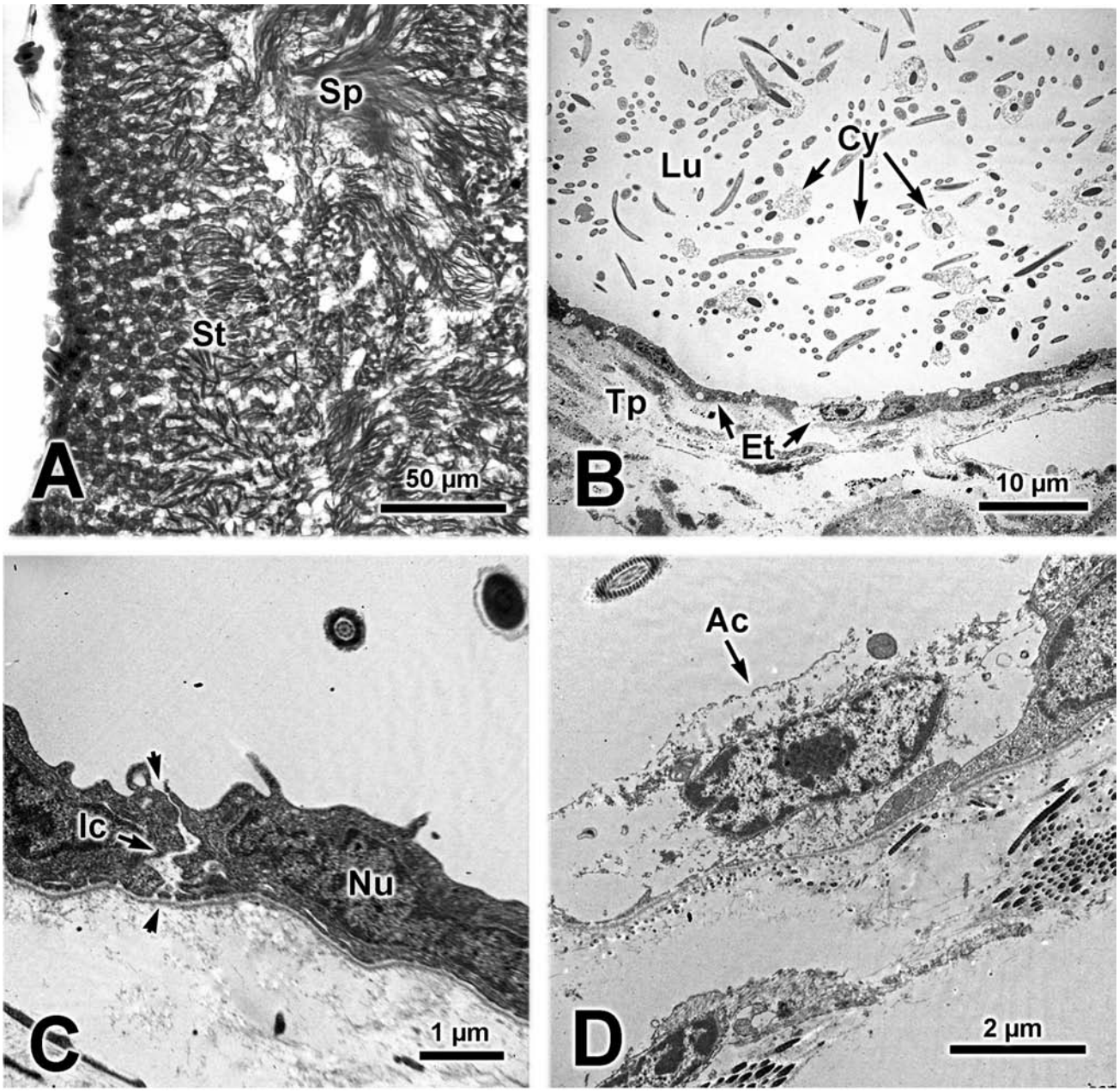

Fig. 7. Testis and rete testis of a 41.1 mm SVL *Scincella lateralis* collected June 6, 2009. (**A**) LM of a paraffin section of the testis stained with hematoxylin and eosin. (**B**) TEM overview of the rete testis with sperm in the lumen. (**C**) TEM of two rete testis cells. (**D**) TEM of an apoptotic rete testis cell. Ac, apoptotic cell; Cy, cytoplasmic bodies around sperm nuclei; Et, epithelium of the rete testis; Ic, intercellular canaliculi; Lu, lumen; Nu, nucleus; Sp, sperm; St, spermatid; Tp, tunica propria.

are associated with masses of cytoplasm (Fig. 7B). The cytoplasm of the cells of the rete testis are devoid of organelles, nuclei are irregular in outline, and the intercellular canaliculi between adjacent cells lack junctional complexes (Fig. 7C). Incidences of apoptosis are found occasionally among the epithelial cells of the rete testis (Fig. 7D).

The ductuli efferentes look similar to those of the April specimen except for presence of some finely granular matter in the lumen (Fig. 8A) and the absence of an apical tight junction between cells, although the open canals are narrow apically and basally (Fig. 8B). In fact, no junctional complexes occur along the length of the intercellular canaliculi, which are widened among much of their length (Fig. 8B). Occasional intracellular spaces occur (Fig. 8B). The finely granular luminal material may represent the cytoplasmic bodies surrounding the sperm nuclei in the rete testis, because these bodies are missing in the ductus epididymis.

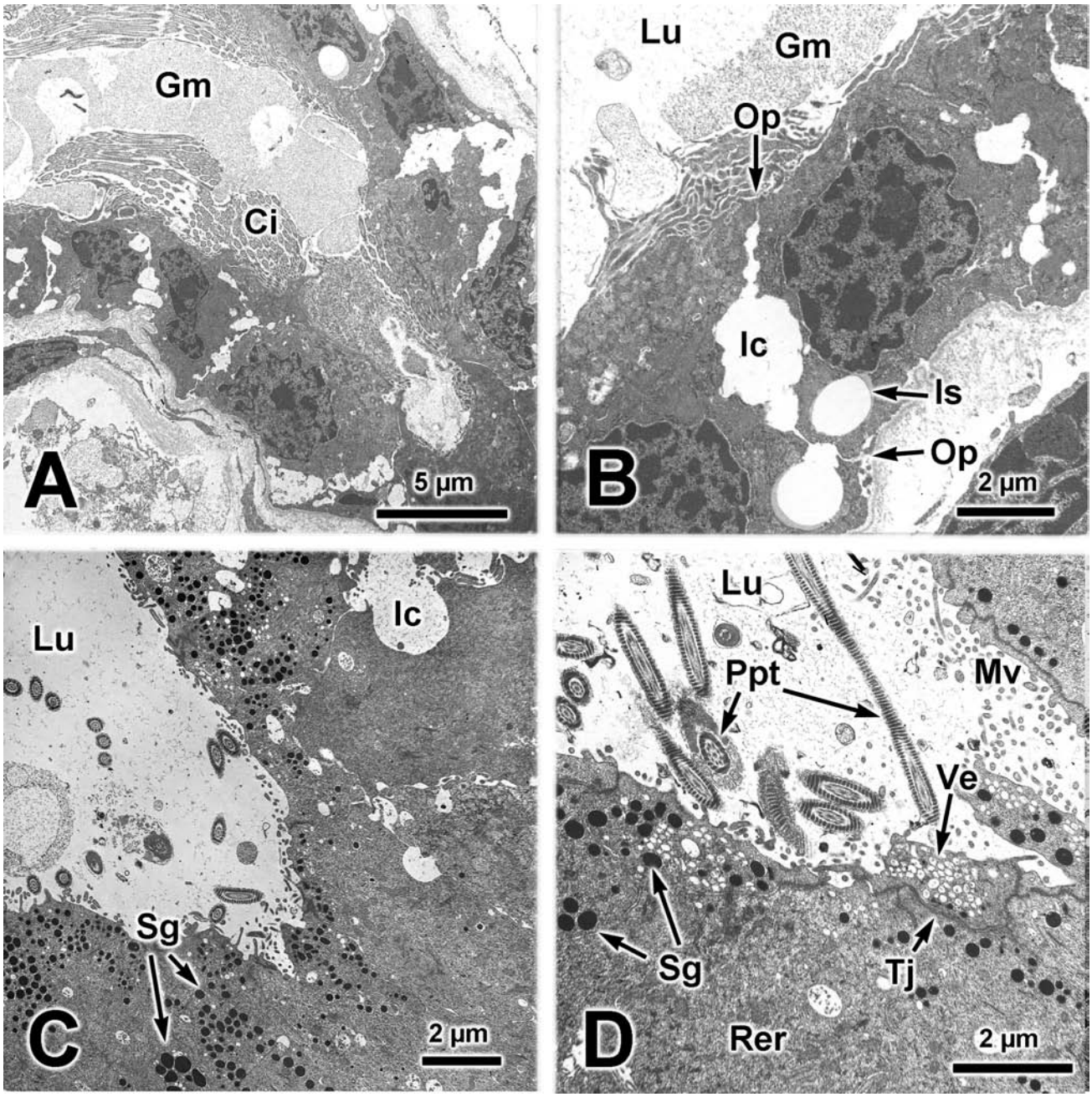

Fig. 8. *Scincella lateralis*, same specimen as used for Figure 7, showing TEMs of the ductuli efferentes and anterior portion of the ductus epididymis. (**A**) Overview of a tubule of ductuli efferentes. (**B**) Ciliated and nonciliated cells of the ductuli efferentes. (**C**) Overview of apical cytoplasm and lumen of the anterior portion of the ductus epididymis. (**D**) Apical secretory granules of the ductus epididymis. Ci, cilia; Gm, granular material; Ic, intercellular canaliculi; Is, intracellular space; Lu, lumen; Mv, microvilli; Op, opening of the intercellular canaliculi; Ppt, principal piece of the cell; Rer, rough endoplasmic reticulum; Sg, secretory granules; Tj, tight junction; Ve, vesicles.

The anterior one-third of the ductus epididymis has a dense cytoplasm characterized by numerous small secretory granules and clear vesicles dorsally (Fig. 8C,D). The intercellular canaliculi still have some widened spaces (Fig. 8C), although not as frequently as in April, and apical tight junctions are extended (Fig. 8D). The luminal areas surrounding sperm are relatively clear except for some fine granular material (Fig. 8D). Rough endoplasmic reticulum still forms a felt-work in the cytoplasm but they are not as clearly delineated as those in the anterior ductus epididymis in April and cannot readily be distinguished from the rest of the cytosol at lower magnifications.

The posterior two-thirds of the ductus epididymis again is more active in producing the secre-

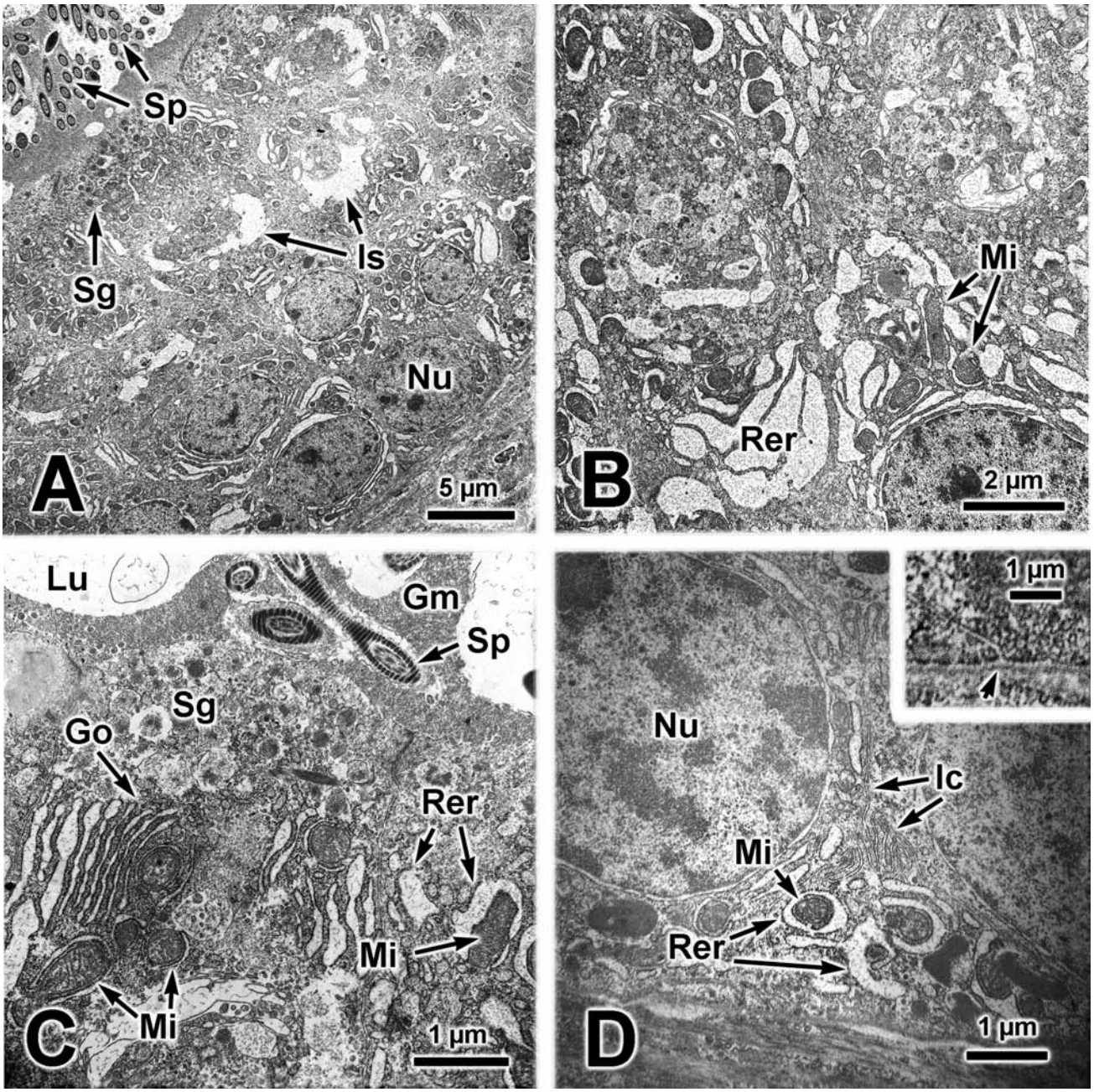

Fig. 9. *Scincella lateralis*, same specimen as used for Figures 7 and 8, illustrating TEMs of the posterior portion of the ductus deferens. (**A**) Overview of several principal cells. (**B**) Supranuclear cytoplasm. (**C**) Luminal border. (**D**) Basal border. Inset: Opening without junctional complexes of intercellular canaliculi basally (unlabeled arrow). Gm, granular material; Go, Golgi complex; Ic, intercellular canaliculi; Is, intracellular space; Lu, lumen; Mi, mitochondria; Nu, nucleus; Rer, rough endoplasmic reticulum; Sg, secretory granules; Sp, sperm.

tions that bathe sperm in the lumen in this region (Fig. 9A). Distended cisternae of Rer, not conspicuous in this region in April, occur supranuclearly (Fig. 9B) and extend to the luminal border where the apical cytoplasm and secretory granules diffuse into the lumen in an apocrine manner (Fig. 9C). Intracellular spaces that appear to lack membranes are also found (Fig. 9A). Some of the cisternae may represent Golgi bodies (Fig. 9C), as granulations appear to be absent, or transitional elements of the Rer or pre-Golgi intermediates (Pavelka and Roth, 2005). Small, dense mitochondria are nearly encircled by cisternae of Rer (Fig. 9B,C), which may be transferring proteins to the mitochondria that they cannot make themselves (Kristic, 1979). These associations continue infranuclearly in the scant cytoplasm between the nuclei and the basement membrane (Fig. 9D).

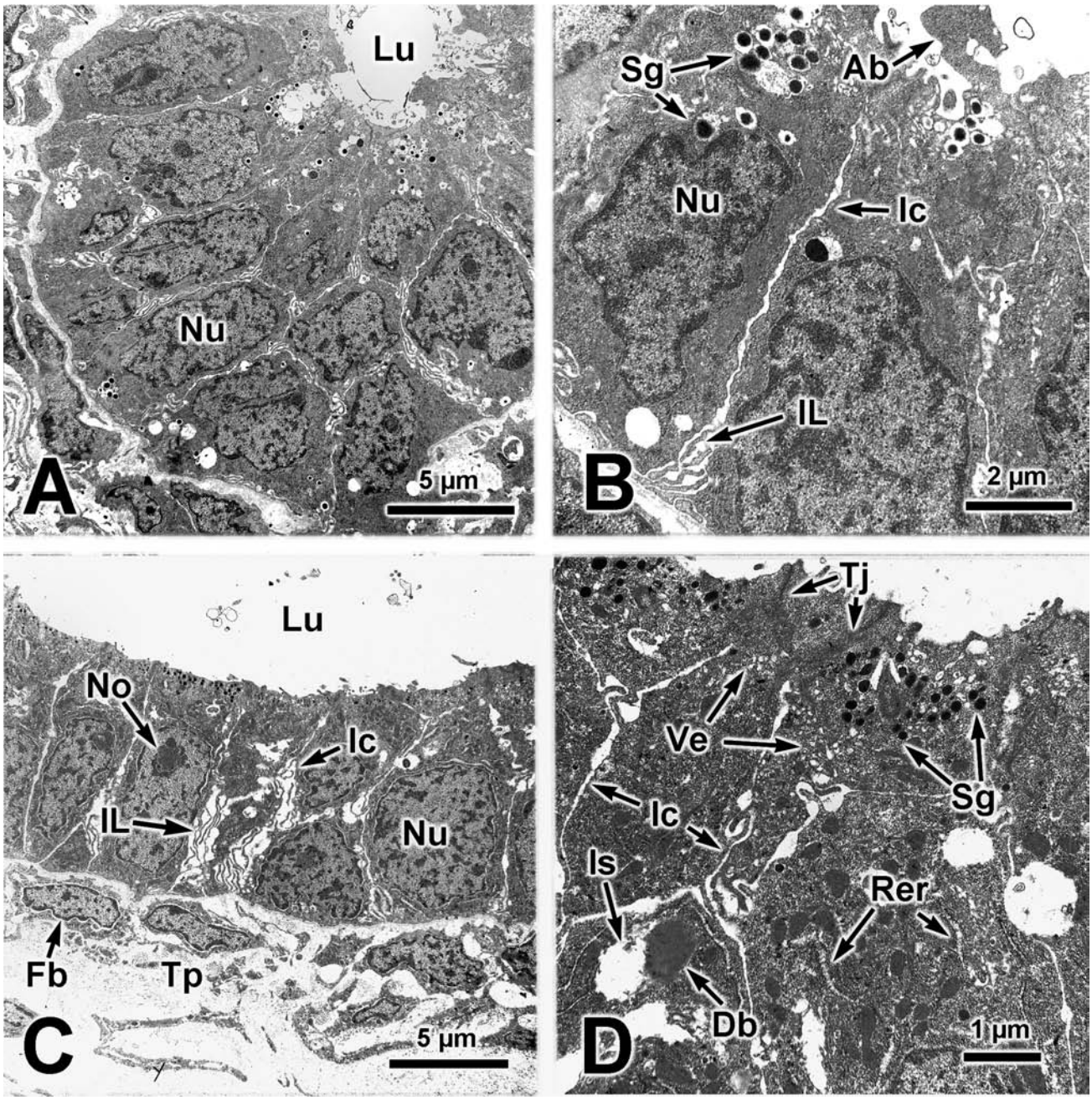

Fig. 10. *Scincella lateralis*. Ductus epididymis from a 33.0 mm SVL specimen collected September 27, 2011 (**A**, **B**) and a 40.5 mm SVL specimen collected October 27, 2006 (**C**, **D**). (A) Overview of principal and basal cells. (B) Several adjacent principal cells with secretory granules. (C) Overview of principal cells. (D) Apical secretory granules and vesicles. Ab, apocrine bleb; Db, dense body; Ic, intercellular canaliculi; IL, intercellular lamellae; Is, intercellular space; Lu, lumen; No, nucleolus; Nu, nucleus; Rer, rough endoplasmic reticulum; Sg, secretory granules; Tj, tight junction; Tp, tunica propria; Ve, vesicles.

Plasma membranes are difficult to detect between adjacent principal cells, but basally we found the narrow intercellular canaliculi opening without junctional complexes into the lamina lucida of the basement membrane (Fig. 9D, inset unlabeled arrow).

In the June specimen, we also examined ultrastructure of the anterior portion of the ductus deferens, the efferent duct that continues past the posterior border of the testis (Supporting Information Fig. S1). The appearance of the ductus deferens is similar to the posterior ductus epididymis (Supporting Information, Fig. S1A). Rough endoplasmic reticulum and intracellular spaces are found supernuclearly (Supporting Information, Fig. S1B) and continue apically to disassociated cytoplasm along the luminal border (Supporting Information, Fig. S1C). Once again, Rer and mitochondria are found in close contact. The associa-

tion between Rer and mitochondria also occurs infranuclearly (Supporting Information, Fig. S1D). As in the posterior ductus epididymis, plasma membranes between adjacent cells are difficult to discern in the anterior ductus deferens.

September

The testes are regressed in the specimens examined from September (Supporting Information, Fig. S2A), and sperm are lacking in the proximal ducts. The ductus epididymis is much reduced in diameter, with epithelial height of about 10 μm (Supporting Information, Fig. S2B). The rete testis does not appear much different than in June, except for the narrow lumen devoid of sperm (Supporting Information, Fig. S2C). Nuclei of the rete testis, however, are even more irregular in shape than in June. Again, junctional complexes between adjacent cells of the rete testis are lacking (Supporting Information, Fig. S2C). The ductuli efferentes also show little variation, although tight junctions, absent apically in June, occur once again as in April (Supporting Information, Fig. S2D). The intercellular canaliculi are not as widened as in April or June, but some intracellular spaces still occur (Supporting Information, Fig. S2D).

No variation was found along the ductus epididymis. The heterochromatic nuclei occupy the bulk of the cytoplasm (Fig. 10A; Supporting Information, Fig. S3A). Rough endoplasmic reticulum is not highly developed, although some secretory granules remain in the apical cytoplasm (Fig. 10B; Supporting Information, Fig. S3B,C). These are typically spherical electron dense particles 0.05–0.25 μm dia, and many are surrounded by flocculent substance that makes it appear the central protein core is surrounded by mucin (Fig. 10B; Supporting Information, Fig. S3B,C). Tight junctions are limited to the apical ends of adjacent plasma membranes, and the intercellular canaliculi remain relatively narrow (Supporting Information, Fig. S3B,C) until basally when they are more convoluted (Supporting Information, Fig. S3D). Lamellae from the principal cells interdigitate with one another basally in the intercellular spaces (Fig. 10B; Supporting Information, Fig. S3B).

October

The testes show more mitotic and meiotic activity, and spermatids are elongating (Supporting Information, Fig. S4A). All of the proximal efferent ducts lack sperm, and the principal cells of the ductus epididymis have cells with heights from 10 to 15 μm (Supporting Information, Fig. S4B). The ductuli efferentes look much the same as in other months (Supporting Information, Fig. S4C), except there is proliferation of Rer in the supranuclear area in some of the nonciliated cells (Supporting Information, Fig. S4D).

As in September, the heterochromatic nuclei of the principal cells of the ductus epididymis largely fill the cytoplasm (Fig. 10C; Supporting Information, Fig. S5A). The intercellular canaliculi are widened among much of their length and contain many interdigitating lamellae from adjacent principal cells (Fig. 10C; Supporting Information, Fig. S5A,B). The intercellular canaliculi once again open without junctional complexes into the basement membrane (Supporting Information, Fig. S5B). Apically, the intercellular canaliculi narrow and form tight junctions at the luminal border (Fig. 10D; Supporting Information, Fig. S5C,D). Narrow filaments of Rer are found in the supranuclear cytoplasm (Fig. 10D; Supporting Information, Fig. S5D). Some cells, but not all, have clusters of small electron dense secretory granules 0.05–0.10 μm as well as small vesicles (Fig. 10D; Supporting Information, Fig. S5D). A summary of the seasonal variation seen in the proximal efferent ducts of *S. lateralis* is given in Table 1.

DISSCUSSION

A significant number of histological works at the LM level have been done on the proximal efferent ducts of squamates, dating back at least to Van der Stricht (1893), who described in the epididymal epithelium of *Z. vivipara* (Lacertidae) secretory structures he named "Vesicules hyalines," later dismissed by Alverdes (1926) as artifacts of poor fixation. Much of the earlier work was reviewed by Fox (1977), and Sever (2010) and Rheubert et al. (2010) provide reviews of more recent literature.

The first paper dealing with the Scincidae was Reynolds (1943) study on eastern North American *P. fasciatus*, usig specimens that mostly came from supply houses that gathered animals from a number of states. Reynolds (1943) reported that a rete testis does not exist but that seminiferous tubules empty into a "small testis outlet duct" that extratesticularly connects with a "longitudinal canal" that gives rise to a number of vasa efferenti (= ductuli efferentes). The ductuli efferentes subsequently empty into the ductus epididymis. A "very short cuboidal epithelium, in which nuclear size is large relative to cell size" forms the lining of both the testis outlet duct and the longitudinal canal, which we interpret to represent the intratesticular rete testis and extratesticular rete testis, respectively, as described herein for *S. lateralis*.

Reynolds (1943) did not mention ciliation in the ductuli efferentes. He noted that the ductus epididymis was not consistent throughout its length; epithelial height was greatest anteriorly, lumen di-

TABLE 1. Variation in the proximal testicular ducts of Scincella lateralis

Location/Structure	April	June	September	October
Rete testis				
Simple squamous epithelium	Yes	Yes	Yes	Yes
Lumen wide, filled with sperm	Yes	Yes	No	No
Sperm associated with cytoplasmic masses		Yes	–	
Absence of junctional complexes		Yes	Yes	
Ductuli efferentes				
Simple cuboidal epithelium with ciliated and non-ciliated cells	Yes	Yes	Yes	Yes
Sperm in lumen	Yes	Yes	No	No
Granular substance in lumen	No	Yes	No	No
Apical coated vesicles	Yes	No	No	No
Apical tight junctions	Yes	No	Yes	Yes
Widened intercellular canaliculi	Yes	Yes	No	No
Intracellular spaces	Yes	Yes	Yes	Yes
Rer conspicuous in some cells	No	Yes	No	Yes
Junctional complexes basally	No	No	No	No
Anterior ductus epididymis				
Sperm in lumen	Yes	Yes	No	No
Granular material abundant in lumen	No	No	No	No
Light and dark cells	Yes	No	No	No
Electron dense secretory granules	Yes	Yes	Yes	Yes
Flocculent ring around secretory granules	No	No	Yes	No
Small clear vesicles apically	No	Yes	No	Yes
Tight junctions apically	Yes	Yes	Yes	Yes
Widened intercellular canaliculi	Yes	Yes	No	Yes
Labyrinthine intercellular canaliculi	No	No	Yes	Yes
Intracellular spaces	Yes	Yes	Yes	Yes
Rer abutting on intercellular canaliculi and intracellular spaces	Yes	No	No	No
Interdigitating lamellae from adjacent plasma membranes	No	No	Yes	Yes
Junctional complexes basally	No	No	No	No
Posterior ductus epididymis				
Sperm in lumen	Yes	Yes	No	No
Granular material abundant in lumen	Yes	Yes	No	No
Light and dark cells	Yes	No	No	No
Dark cells with Rer abundant to luminal border	Yes	–	–	–
Tight junctions apically	Yes/no	No	No	No
Luminal border obscure/apocrine secretion	No	Yes	No	No
Intracellular spaces more numerous than anteriorly	Yes	Yes	No	No
Distended Rer perinuclear	No	Yes	No	No
Rer abutting on intercellular canaliculi and intracellular spaces	Yes	No	No	No
Mitochondria surrounded by Rer cisternae	No	Yes	No	No
Junctional complexes basally	No	No	No	No
Basal opening of intercellular canaliculi widened	Yes	No	No	No

ameter increased posteriorly, and overall tubule diameter increases slightly posteriorly.

Badir (1958) studied the urogenital organs of two Egyptian skinks, *S. scincus*, a fossorial desert lizard with an attenuated body and short limbs, and *C. ocellatus*, a typical skink preferring less arid habitats. These two species both have multiple rete testis outlets from the seminiferous tubules; Badir (1958) reported six in *S. scincus* and nine in *C. ocellatus*.

In *S. scincus*, the rete testis tubules after exiting the testis open into a longitudinal canal that, like the rete tubules, is composed of "low epithelial cells with oval or round nuclei." The longitudinal canal opens "from time to time" into the ductuli efferentes, which can be distinguished by their cilia and more columnar epithelium. The ductuli efferentes shorten as they approach the ductus epididymis, which is easily distinguished by its pseudostratified epithelium, outer layer of smooth muscle, and large diameter which increases posteriorly. No line of demarcation exists between the ductus epididymis and ductus deferens.

In *C. ocellatus*, Baird (1958) reported that the two most cranial seminiferous tubules extend out of the capsule of the testis into the mesorchium to form the anterior-most rete testes. The initial nine rete testes branch repeatedly in the mesorchium forming a network until 12 branches connect to ducts Badir called the Verbindungsstück. Each rete testes branch has its own Verbindungsstück. The flat epithelium of the rete testes contrasts with simple columnar epithelium of the Verbindungsstück, which has some ciliated cells. The Verbindungsstück is also characterized by possessing secretory granules not just seasonally, as in other ducts, but throughout the year.

The Verbindungsstück in turn open into the ductuli efferentes, the main difference being the lack of secretory granules and more regular ciliation in

the latter. Badir (1958) does not discuss the transition from the ductuli efferentes into the ductus epididymis, which, we presume is therefore the same as for *S. scincus*. Badir notes that the proximal ducts of the two skinks are indeed quite different from one another and that *C. ocellatus* resembles earlier descriptions by Alverdes (1928) for certain snakes. Clearly these descriptions are quite different from the findings of Reynolds (1943) on *E. fasciatus* and our findings on *S. lateralis*, in terms of the number of rete testis tubules and the presence of a longitudinal canal and a Verbindungsstück. The authors hope for an opportunity to examine testicular ducts of *S. sincus*, *C. ocellatus*, and additional species of skinks in the future.

The most comprehensive review of epididymal histochemistry in squamates was by Dufaure and Saint Girons (1984), who examined the ductus epididymides of 89 species of squamates, representing 72 genera and 18 families. Ten species of skinks were included from Africa, Asia, Australia, and/or New Zealand: *Abepharus* (= *Cryptoblepharus*) *boutonii, Chalcides mionecton, C. ocellatus, C. polylepis, Eumeces schneideri, Egerenia* sp., *Leiolopisma fuscum, L. zelandica, Lygosoma* (= *Ctenotus*) *taeniolatus,* and "*Mabuia semifasciata*" (this is not a currently valid name for any skink; what species it represents is unknown to us). They found five types of secretory activity in the ductus epididymis of the squamates examined, ranging from possession of large secretory granules to no granules at all. The skinks were characterized by a state in which granules are not distinct but the cytoplasm is dense and heterogeneous and a secretory product can be found in the lumen. When mapped on molecular and morphological phylogenies of squamates, Trauth and Sever (2011) found that this condition is the ancestral condition for squamates. The presence study confirms that secretory granules are indeed hard to detect with LM in *S. lateralis*, but small electron dense granules are observed with TEM in the apical cytoplasm in all samples, although they are larger and more numerous in active epididymides.

The first ultrastructure study on the testicular ducts was by Mesure et al. (1991) who studied the fine structure of the ductus epididymis of the Eurasian lizard *Z. vivipara* (Lacertidae), in which they recognized four regions: caput, proximal corpus, distal corpus, and cauda, but only small differences were distinguished. Mesure et al. (1991) recognized only two types of cells, secretory principal cells and nonsecretory basal cells. The secretory cells undergo an annual cycle with 10 stages. After production of the protein secretion that binds with the heads of sperm, the secretory cells undergo a dramatic involution that results in the elimination of the greatest part of the cytoplasm. The cauda is nonsecretory and is a region for sperm storage.

Meeran et al. (2001) studied ultrastructure of the ductus epididymis of *C. versicolor* (Agamidae) collected during the breeding season in India, extending observations made by Averal et al. (1992) using LM. They found the epididymis differentiated into four zones comparable to the mammalian initial segment, caput, corpus, and cauda. Seven cell types were recognized: principal, narrow, apical, clear, dark, basal, and halo, with only slight differences from mammals in their distribution and secretory activity.

Desantis et al. (2002) reported that the ductus epididymis has a caput (cranial), corpus (middle), and cauda (caudal) in the southern European lacertid *P. sicula*. These areas show a gradual decrease in height of secretory cells and a corresponding increase in luminal secretory granules and accumulation of sperm. Like Mesure et al. (1991), Desantis et al. (2002) recognized only two types of cells, secretory principal cells and nonsecretory basal cells.

The primary aim of the study of Desantis et al. (2002) was to characterize the oligosaccharide sequences of glycoconjugates using lectin histochemistry. Basal cells show weak lectin reactivity with one glycan, and the secretory cells along the length of the epididymis have 12 lectins binding to the secretions, with some regionalization. However, the function of the glycoconjugates in the epididymal secretory granules was not determined, but perhaps they produce an environment for sperm maturation and/or storage, as known for mammals (Acott and Hoskins, 1981). Although Desantis et al. (2002) is the most comprehensive lectin study on squamates, much comparative work, especially in mammals, occurs on lectin histochemistry in the efferent ducts (e.g., Arya and Vanha-Perttula, 1986; Arenas et al., 1998).

Akbarsha et al. (2006a,b) conducted a TEM study of the ductus epididymis in the Indian agamid *S. ponticeriana*. Like Meeran et al. (2001), they found that the ductus epididymis is divided into four zones corresponding to the initial segment, caput, corpus, and cauda of the mammalian ductus epididymis. Six cells types occur. The principal cells constitute 80–85% of the epithelium of the initial segment, caput, and corpus but only 70% of the cauda. Narrow cells (1–2%) and apical cells (0.5–1%) are found only in the initial segment, and clear cells (10–20%) are limited to the cauda. Basal cells (10–20%) and intraepithelial leukocytes (2–5%) are present throughout the ductus epididymis. The initial segment, caput, and corpus are associated with secretion, absorption, and endocytosis, whereas the cauda is concerned with absorption, endocytosis, and storage.

Finally, Akbarsha et al. (2007) extended their work on the efferent ducts of *S. ponticeriana* to include the most proximal ones, which they referred to simply as the "efferent ductules" and

which appear to be limited to the ductuli efferentes. They reported that six to eight ductules originate from an extratesticular rete testis and pass thru the mesorchium into the caput of the epididymis. The epithelium is pseudostratified with columnar ciliated or non-ciliated cells, along with scattered basal cells and "dark cells"; the latter perhaps representing degenerating nonciliated cells. The columnar nonciliated cells contain apical vesicles involved in uptake of material and also, like cauda cells in the ductus epididymis, are involved in spermiophagy. Spermiophagy has been well known for many years in the testes and the efferent ducts of mammals (e.g., Hoffer et al., 1973; Cooper and Hamilton, 1977).

Ferreira et al. (2009) studied seasonal variation in the histology and ultrastructure of the ductus epididymis of *T. itambere* from southeastern Brazil. Their findings were rather brief. Two types of cells, columnar secretory cells and small wedge-shaped basal cells occur. Secretory granules and epithelium height increases are initiated before spermiation and apparently secretory activity is maintained while sperm pass through the ductus epididymis, followed by shortening of epithelial height and cessation of secretory activity. Ferreira et al. (2009) did not mention the rete testis or ductuli efferentes.

Rheubert et al. (2010) described seasonal variation of the proximal testicular ducts in *H. turcicus* from southeastern Louisiana. They found a single rete testis that divides into three to four ductuli efferentes that drain into the cranial end of the ductus epididymis. The rete testis is squamous but not as thin as in *S. lateralis* and tight junctions occur apically, unlike *S. lateralis*. The rete testis of *H. turcicus* often has widened intercellular canaliculi. Also, scattered rete cells are ciliated, a condition also noted in some birds and mammals (Robaire and Hermo, 1988; Aire, 2007), in which a rete cell may bear a single cilium whereas multiple cilia can occur on rete cells of *H. turcicus*.

The ductuli efferentes of *H. turcicus* is very similar to that of *S. lateralis*. The only notable differences are the presence of smooth endoplasmic reticulum and more lysosomic activity in *H. turcicus*. Also, as reported for *S. ponticeriana* by Arkbasha et al. (2007), evidence of spermiophagic activity occur in *H. turcicus*.

The ductus epididymis of the two lizards also shows some similarities. No regionalization was noted in *H. turcicus,* but the active ductus epididymis is characterized by widened intercellular canaliculi and abundant Rer. Small electron-dense secretory granules are found apically and some persist when the ductus epididymis is inactive, just like in *S. lateralis*.

Ultrastructural descriptions of the proximal testicular ducts of snakes are limited to the natricine *S. pygaea* (Sever, 2010) and elapid *P. platurus* (Sever and Freeborn, 2012) and some brief observations on the viper *A. piscivorus* (Trauth and Sever, 2011). The rete testis and ductuli efferentes of snakes are similar to those described for *H. turcicus* and *S. lateralis*, although the snakes appear to possess more coated vesicles and endosomes. The rete testis, and especially the ductuli efferentes of *P. platurus*, has widened intercellular canaliculi and intracellular spaces similar to those found in *S. lateralis*.

The ductus epididymis again shows the most variation. The ductus epididymis in snakes is pseudostratified with columnar principal cells and basal cells, but in addition, *P. platurus* has mitochondria-rich apical cells. The principal cells in *S. pygaea* and *A. piscivorus* lack secretory granules but the epithelium is very vesicular. Secretion is by a constitutive pathway in which small vesicles and a flocculent material are released via a merocrine process or through the formation of apocrine blebs. In *P. platurus*, however, small biphasic secretory granules occur in the apical cytoplasm. In addition, occasionally a single cilium is found on a principal cell.

The intercellular canaliculi in *S. pygaea* are very narrow, but in *P. platurus,* the intercellular spaces are even more widened and interconnected than in the active ducts of *S. lateralis*. This cytology is very similar to the mammalian ductus deferens as described by Hermo et al. (2002), who suggested that the intracytoplasmic spaces and dilated spaces between adjacent cells are areas of water accumulation and transport. In both *P. platurus* and *S. lateralis*, tight junctions seal the apical border but junctional complexes between cells are absent elsewhere, and the intercellular canaliculi open freely into the basement membranes. Recent work on mammals and birds indicate that aquaporin (AQP) channels are involved in water transport from the lumen into the epithelium of testicular ducts (e.g., Badran and Hermo, 2002; Zaniboni et al., 2004; Huang et al., 2006). As noted by Sever and Freeborn (2012) investigation of AQPs in the male reproductive tracts of reptiles, is an area ripe for investigation.

Some comparisons of ultrastructural characters between the squamates studied so far are presented in Supporting Information, Table S2. The lack of data and comparable detail make any conclusions premature, but the hypothesis that the proximal ducts, especially the ductus epididymis, will show significant variation from other squamates is supported. We do not believe we are at the stage, however, where ancestral character states that would be phylogenetically informative can be assigned for squamates. For example, the presence of ciliated epithelium in the ductuli efferentes and of pseudostratified epithelium in the ductus epididymis are ancestral states for amniotes as a whole and thus symplesiomorphic for squamates. The presence of widened intercellu-

lar canaliculi in the ground skink, *S. lateralis*, and the Yellow-bellied Sea snake, *P. platurus*, should be considered convergence and not retention of an ancestral character. Both the skink and the sea snake are in highly derived clades that lack, in most phylogenies, a recent common ancestor (Conrad, 2008; Vidal and Hedges, 2009).We need to get busy and examine testicular ducts of additional species of squamates.

Finally, some comments on the ductus deferens. In squamates, the ductus deferens is usually described as rather uniform in structure along its length and is the acknowledged organ of sperm storage (Fox, 1952). Anatomical studies on the ductus deferentia of squamates have been limited to gross morphology and LM except for the ultrastructure study by Sever (2004) on the snake *S. pygaea*, by Akbarsha et al. (2005) on the lizard *S. ponticeriana*, and brief observations on *A. piscivorus* (Siegel et al., 2009; Trauth and Sever, 2011) and *P. obsoletus* (Trauth and Sever, 2011). Also, the various modifications at the junction of the ductus deferens and ureter have received some attention (Siegel et al., 2009; Trauth and Sever 2011), although a thorough ultrastructural study of this region is lacking for squamates.

Aire (2007) described the testicular ducts of birds and noted that the avian ductus epididymis and ductus deferens "are merely different segments of the same organ, an organ equivalent to, but not as grossly structured as the epididymis in mammals." He proposed calling the functional unit formed by the ductus deferens and ductus epididymis the "epididymal duct unit." In these regards, we find the similarity between the posterior ductus epididymis and the anterior ductus deferens that we found in the June *S. lateralis* most interesting. The observations can also be related to the findings of Jones (1998, 2002) that reptilian and avian sauropsids actually lack a ductus deferens and the entire duct from the ductuli efferentes to the ureter should be called the ductus epididymis. Again, more comparative anatomical, physiological, and developmental work on all portions of the sperm ducts of squamates is needed.

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