JOURNAL OF MORPHOLOGY 273:324–336 (2012)

Observations on the Anterior Testicular Ducts in Snakes With Emphasis on Sea Snakes and Ultrastructure in the Yellow-Bellied Sea Snake, *Pelamis platurus*

David M. Sever* and Layla R. Freeborn

Department of Biological Sciences, Southeastern Louisiana University, Hammond, Louisiana 70402

ABSTRACT The anterior testicular ducts of squamates transport sperm from the seminiferous tubules to the ductus deferens. These ducts consist of the rete testis, ductuli efferentes, and ductus epididymis. Many histological and a few ultrastructural studies of the squamate reproductive tract exist, but none concern the Hydrophiidae, the sea snakes and sea kraits. In this study, we describe the anterior testicular ducts of six species of hydrophiid snakes as well as representatives from the Elapidae, Homolapsidae, Leptotyphlopidae, and Uropeltidae. In addition, we examine the ultrastructure of these ducts in the yellow-bellied Sea Snake, *Pelamis platurus*, only the third such study on snakes. The anterior testicular ducts are similar in histology in all species examined. The rete testis is simple squamous or cuboidal epithelium and transports sperm from the seminiferous tubules to the ductuli efferentes in the extratesticular epididymal sheath. The ductuli efferentes are branched, convoluted tubules composed of simple cuboidal, ciliated epithelium, and many species possess periodic acid-Schiff+ granules in the cytoplasm. The ductus epididymis at the light microscopy level appears composed of pseudostratified columnar epithelium. At the ultrastructural level, the rete testis and ductuli efferentes of *P. platurus* possess numerous small coated vesicles and lack secretory vacuoles. Apocrine blebs in the ductuli efferentes, however, indicate secretory activity, possibly by a constitutive pathway. Ultrastructure reveals three types of cells in the ductus epididymis of *P. platurus*: columnar principal cells, squamous basal cells, and mitochondria-rich apical cells. This is the first report of apical cells in a snake. In addition, occasional principal cells possess a single cilium, which has not been reported in reptiles previously but is known in some birds. Finally, the ductus epididymis of *P. platurus* differs from other snakes that have been studied in possession of apical, biphasic secretory vacuoles. All of the proximal ducts are characterized by widening of adjacent plasma membranes into wide intercellular spaces, especially between the principal cells of the ductus epididymis. Our results contribute to a larger, collaborative study of the evolution of the squamate reproductive tract and to the potential for utilizing cellular characters in future phylogenetic inferences. J. Morphol. 273:324–336, 2012.

KEY WORDS: squamata; Hydrophiidae; rete testis; ductuli efferentes; ductus epididymis; histology; ultrastructure

INTRODUCTION

The Hydrophiidae contains the sea snakes and sea kraits, and the family is one of five evolutionary lineages of snakes that have adapted to marine environments (Lillywhite et al., 2008). The oviparous sea kraits, *Laticauda* (eight species, subfamily Laticaudinae), *Cerberus rnychops* (Homolapsidae), and certain natricine colubrids (such as *Nerodia clarkii*) have retained connections to terrestrial life. The viviparous "true" sea snakes (some 53 species in 16 genera, subfamily Hydrophiinae) and *Acrochordus granulatus* (Acrochordidae) are fully marine. No general agreement exists on the relationship between the hydrophiine sea snakes and sea kraits. The monophyly of hydrophiine sea snakes, however, is supported by molecular (Cadle and Gorman, 1981; Slowinksi et al., 1997; Keogh et al., 1998; Keogh, 1998; Lukoschek and Keogh, 2006) and morphological (Voris, 1977; Gopalakrishnakone and Kochva, 1990) data.

The greatest hydrophiine sea snake diversity is found in the waters of Malaysia, the Indonesian archipelago, and northern Australia. Thirty-two species, roughly 60% of hydrophiine sea snakes, are found in Australia alone. The Yellow-bellied Sea Snake (*Pelamis platurus*) has the widest distribution of any snake, extending from eastern Africa to the western coast of Central and South America. Sea kraits occur in the waters of

Layla R. Freeborn is currently at Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, Louisiana 70118

Contract grant sponsor: National Science Foundation; Contract grant number: DEB-0809831.

*Correspondence to: David M. Sever, Department of Biological Sciences, Southeastern Louisiana University, Hammond, LA 70402. E-mail: dsever@selu.edu

Received 23 June 2011; Revised 31 August 2011; Accepted 1 September 2011

Published online 24 October 2011 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/jmor.11025

TABLE 1. Specimens utilized in this study

Family/Species	Coll number	SVL (cm)	Date coll	Locality
Acrochoridae				
Acrochordus granulatus	FMNH 202791	35.5	20 September 1975	Bohol Islands, Philippines
Elapidae				
Micurus tener	UTAR 32601	46.6	24 June 1992	Valverde County, Texas
Micurus tener	UTAR 36753	55.2	18 March 1958	Liberty County, Texas
Micurus tener	UTAR 36758	[a]	31 March 1956	Harris County, Texas
Homolapsidae				
Cerberus rynchops	FMNH 202757	62.5	19 September 1975	Bohol Islands, Philippines
Hydrophiidae: Laticaudinae				
Laticauda colubrina	FMNH 202798	92.5	15 September 1975	Philippines
Laticauda colubrina	FMNH 202801	99.5	11 September 1975	Philippines
Hydrophiidae: Hydrophiinae				
Aipysurus eydouxii	FMNH 199455	61.5	8 March 1975	Johore, West Maylasia
Ephalophis greyi	FMNH 212349	65.0	16 February 1980	Western Australia
Ephalophis greyi	FMNH 212355	62.5	8 March 1980	Western Australia
Hydrophis lamberti	FMNH 202922	80.0	11 September 1975	Gigantes Islands, Phillipines
Hydrophis ornatus	FMNH 257233	80.0	24 May 1998	Sabah, Maylasia
Hydrophis ornatus	FMNH 257236	84.0	24 May 1998	Sabah, Maylasia
Pelamis platurus	SLU 0133	47.6	10 July 2009	Guanacaste, Costa Rica
Pelamis platurus	SLU 0135	52.5	10 July 2009	Guanacaste, Costa Rica
Pelamis platurus	SLU 0138	56.5	10 July 2009	Guanacaste, Costa Rica
Leptotyphlopidae				
Leptotyphlops dulcis	FMNH 40956	11.2	10 April 1943	McLennan County, Texas
Leptotyphlops dulcis	FMNH 40961	10.8	10 April 1943	McLennan County, Texas
Uropeltidae				
Teretrurus sanguineus	FMNH 265872	16.0	16 April 1972	India

[a]Missing data.

southeastern Asia, the southwestern Pacific islands, New Guinea, Northern Australia, and the Philippines (Minton, 1975).

Timing of reproduction and annual reproductive cycles are highly varied among and within sea snakes and other marine species. Three sympatric species of marine snakes from the Philippines have different seasonal patterns (Gorman et al., 1981). The homalopsid *Cerberus rynchops* is loosely seasonal, the acrochordine *A. granulatus* is highly seasonal, and *Laticauda colubrina* is aseasonal. However, *A. granulatus* breed seasonally in the Straits of Mallaca (Lemen and Voris, 1981), and *L. colubrina* reproduce seasonally in Fiji (Guinea, 1981, 1986). The reproductive cycles of *Aipysurus laevis* from the Great Barrier Reef (Burns, 1984), *Enhydrina schistosa* from Malaysia (Voris and Jayne, 1979), and several other species of marine snakes from Malaysia (Lemen and Voris, 1981) have also been described. Details of mating and courtship of sea snakes are unknown.

Since Morgera (1905) first described the proximal efferent ducts of snakes in the genera *Coluber* and *Elaphe* (Colubridae), a number of histological studies of snake reproductive tracts have appeared (reviewed by Trauth and Sever, 2011). This article is the first report on histology of the anterior testicular ducts of species within the Hydrophiidae. We also compare our findings on hydrophiids to new observations on histology of these ducts in the marine snakes *A. granulatus* and *C. rynchops* as well as terrestrial snakes *Leptotyphlops dulcis* (Leptotyhlopidae), *Teretrurus sanguinus* (Uropeltidae), and *Micurus tener* (Elapidae). The Leptotyphlopidae are generally considered one of the most basal groups of snakes. The Uropeltidae is in the clade Henophidia, a group of snakes that form the sister group of the most advanced snakes, the Caenophidia, which includes all other species studied herein except for *L. duclis*. The Elapidae are considered the sister taxon of Hydrophiidae (Burbrink and Crother, 2011). Indeed many authorities consider Hydrophiidae to be a subfamily of Elapidae (Scanlon and Lee, 2011).

In addition, only two articles have described the ultrastructure of the proximal testicular ducts of snakes, Sever (2010) on the natricine snake *Seminatrix pygaea* and Trauth and Sever (2011) on the viperine snake *Agkistrodon piscivorus*. In this article, we present observations from transmission electron microscopy (TEM) on ultrastructure of the rete testis, ductuli efferentes, and ductus epididymis of *P. platurus*.

MATERIALS AND METHODS

Species Utilized

Specimens of *P. platurus* were collected specifically for this study and were deposited in the vertebrate collections at Southeastern Louisiana University (SLU). The remaining specimens came from collections at the Field Museum, Chicago, IL (FMNH) and the University of Texas, Arlington (UTAR). Table 1 summarizes source, snout-vent length (SVL), collection date, and locality information. Detailed locality data are unknown for some specimens.

Collection and Tissue Preparation

Specimens of *P. platurus* were collected July 10, 2009, ~12 km south of Playa del Coco in Golfo de Papagayo (Guanacaste, Costa Rica). Individual snakes were collected in dip nets and placed in a large plastic storage container filled with sea water. Specimens were euthanized within 12 h of capture by lethal injection (3–5 ml) of 10% sodium pentobarbital in 70% ethanol. This procedure was approved by the Institutional Animal Care and Use Committee of SLU, Hammond, LA. 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 light microscopy. The 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) for electron microscopy. All required permits for the collection, euthanasia, and dissection of *P. platurus* were obtained from the Ministerio del Ambiente y Energía of the Costa Rican government.

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 h. Tissues were then embedded in paraffin blocks that were allowed to harden before 10-μm-thick sections were cut with a rotary microtome (RMC Instruments, Tucson, AZ) and affixed to albuminized 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). Three-dimensional reconstructions of anterior testicular ducts of *P. platurus* were made with BioVis3D (Montevideo, Uruguay) with 200 μm between serial sections.

Tissues for TEM were rinsed in deionized water, postfixed in 2% osmium tetroxide, dehydrated through a graded series of ethanol (same as light microscopy), cleared in propylene oxide, and embedded in epoxy resin (Embed 812, Electron Microscopy Sciences, Hatfield, PA). Plastic sections were cut using a Reichert ultramicrotome (Reichert Microscope Services, Depew, NY) and DiATOME (Biel, Switzerland) diamond knives at 1 μm and 70 nm. Toluidine blue was used to stain 1-μm-thick sections. Sections of 70 nm 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, 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).

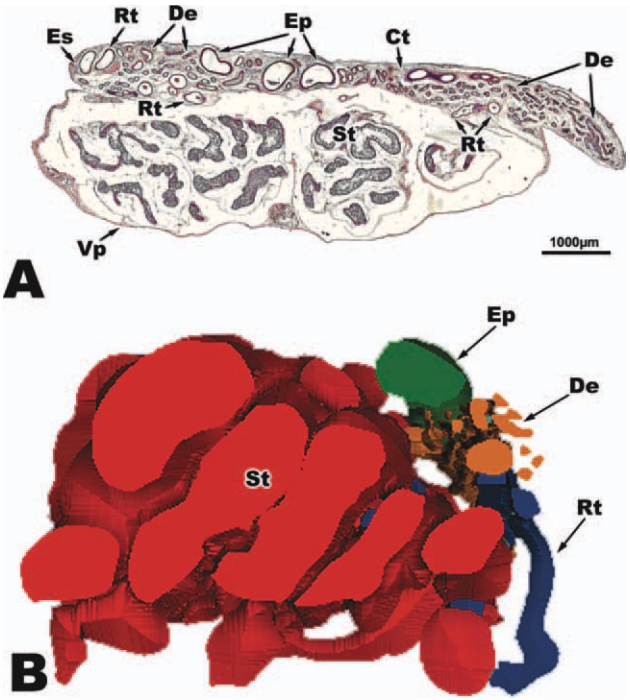

Fig. 1. (**A**) Light micrograph of a midsagittal section through the testis of *E. greyi* (FMNH 212349) showing the overall relationships of the anterior testicular ducts to the testis. (**B**) Three-dimensional reconstruction of a transverse section through the anterior testicular ducts and adjacent testis of *P. platurus* (SLU 0138). Ct, connective tissue; De, ductuli efferentes; Ep, ductus epididymis; Es, epididymal sheath; Rt, rete testis; St, seminiferous tubules; Vp, visceral pleuroperitoneum.

RESULTS

Histology

The general organization of the testis and anterior testicular ducts is shown in sagittal section in Figure 1A and as a three-dimensional reconstruction of a transverse section in Figure 1B. Histological sections through these regions in the sea krait and sea snakes are shown in Figure 2, and other species are illustrated in Figure 3. Overall, the anterior testicular ducts show little variation at the light microscopy level among the species examined, and the divisions among ducts are readily recognized in all species.

The visceral pleuroperitoneum lies superficial to the thin connective tissue lining that covers the seminiferous tubules and the ducts. The rete testis extends from the seminiferous tubules into the extratesticular epididymal sheath that is continuous with the covering of the testis and encases the ductuli efferentes and epididymis (Fig. 1A). The adrenal gland and numerous blood vessels are also located within the epididymal sheath.

All of the ducts have eosinophilic cytoplasm. Histochemical reactions were muted in museum specimens from FNNH and UTAR, many of which had been in ethanol storage for >30 years. Epithelia of all ducts of *P. platurus* freshly collected for this study, however, react positive for PAS (general carbohydrates), especially the rete testis. Epithelia are negative for AB at pH 2.5 (carboxylated gylcosaminoglycans) and show only a slight response with BB (proteins). In the other species, PAS+ granules occur in the ductuli efferentes of *Aipysurus eydouxii*, *Ephalophis greyi*, *Hydrophis lamberti*, *Hydrophis ornatus*, and *Teretrurus sanguinensis* whereas *M. tener* lacks the granules but has a PAS+ luminal border on the ductuli

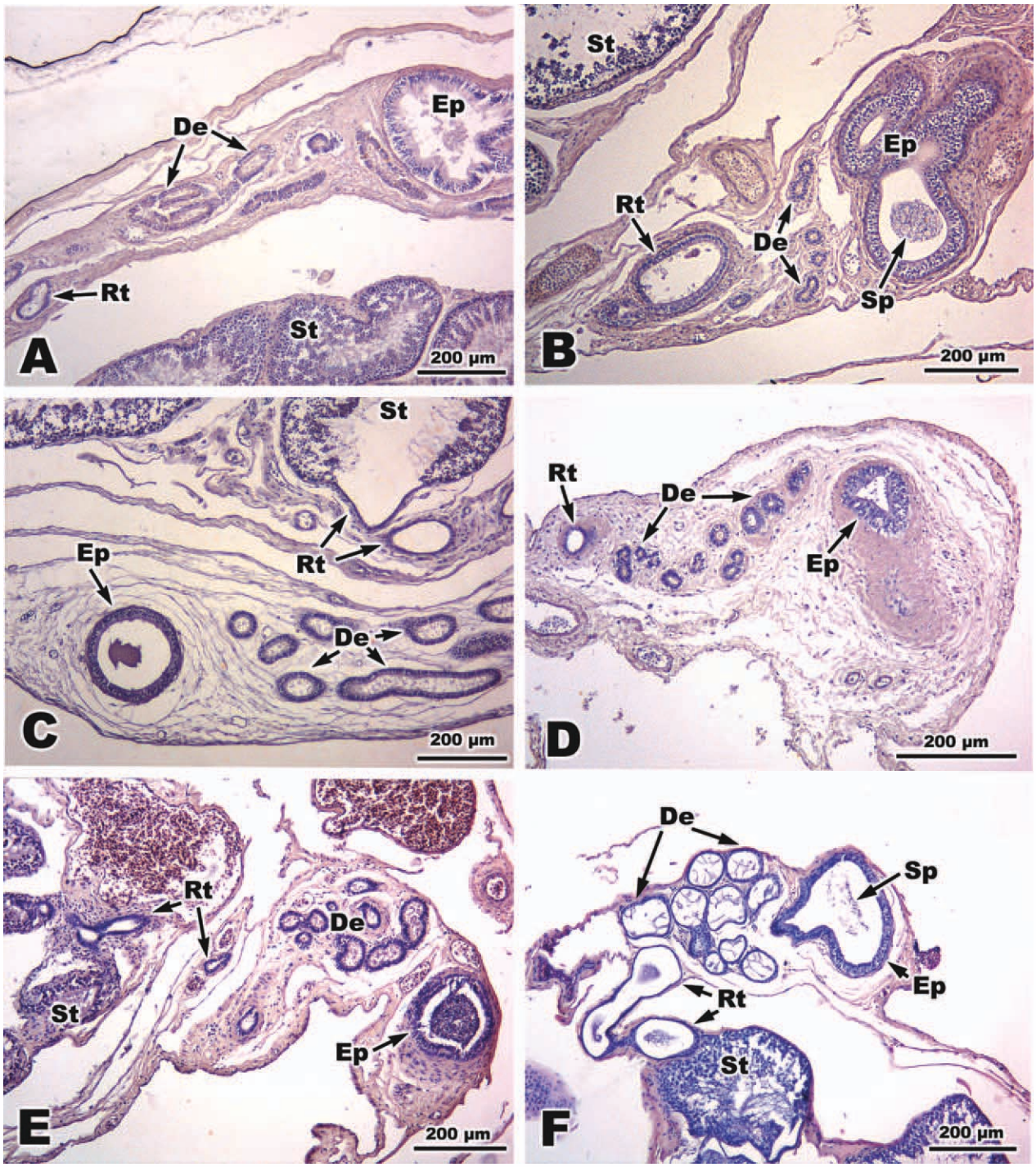

Fig. 2. Light micrographs of the anterior testicular ducts of sea snakes. (**A**) *L. colubrina* (FMNH 202798). (**B**) *A. eydouxii* (FMNH 199455). (**C**) *E. greyi* (FMNH 212355). (**D**) *H. lamberti* (FMNH 202922). (**E**) *H. ornatus* (FMNH 257233). (**F**) *P. platurus* (SLU 138). De, ductuli efferentes; Ep, ductus epididymis; Rt, rete testis; Sp, sperm; St, seminiferous tubules.

efferentes. Other histochemical reactions cannot be considered positive in these species.

Sperm occur in the ductus epididymis of all specimens except *C. rynchops*, *E. greyi*, *H. lamberti*, and *L. dulcis*. When sperm are present in the ductus epididymis, they are also apparent in the ductuli efferentes. Sperm in the ductuli efferentes, however, are sometimes difficult to discern because of the small diameter of those tubules and the presence of numerous cilia. Sperm are found in the rete

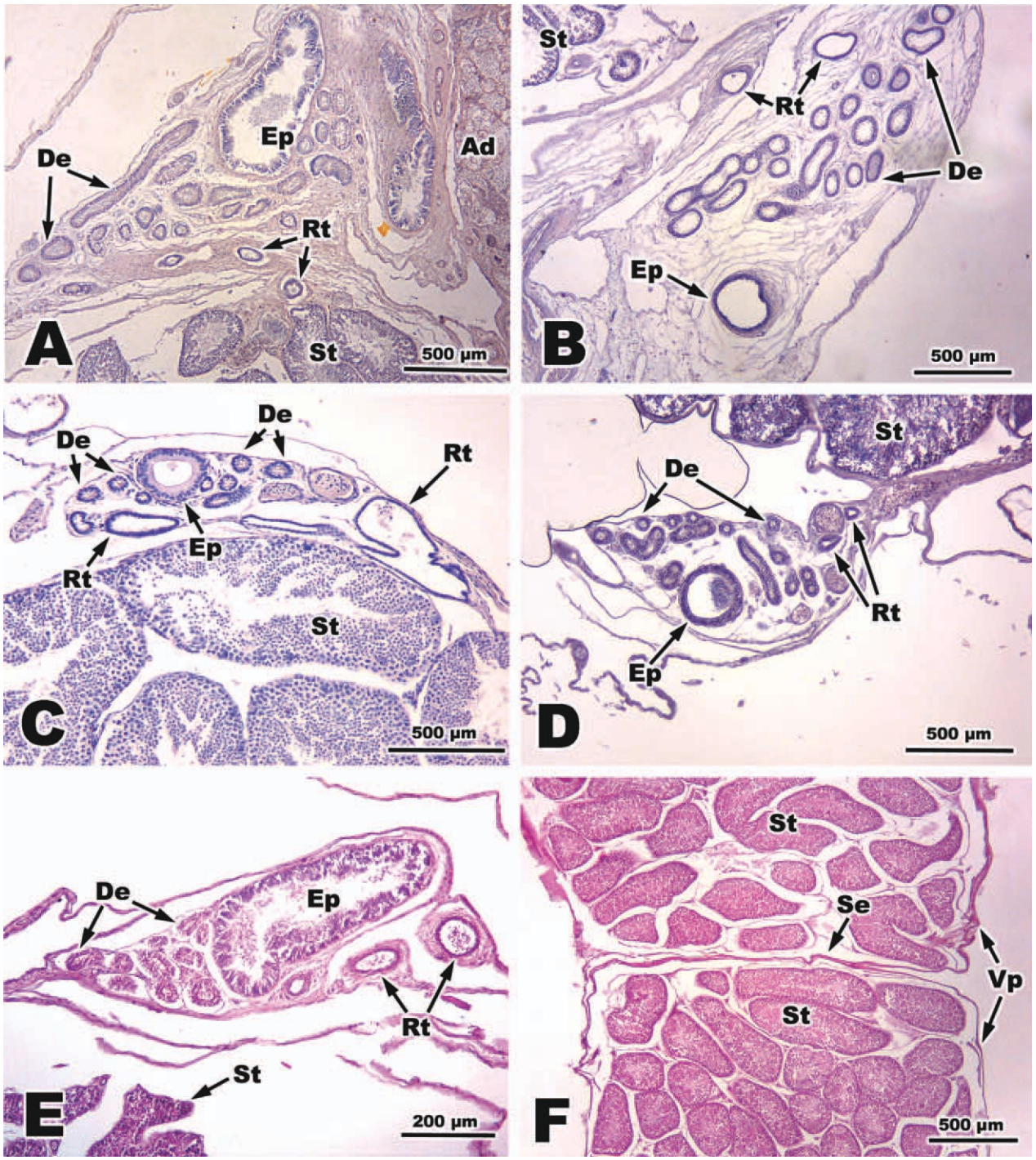

Fig. 3. Light micrographs of the anterior testicular ducts of snakes. (**A**) *A. granulatus* (FMNH 202791). (**B**) *C. rynchops* (FMNH 202757). (**C**) *L. dulcis* (FMNH 40956). (**D**) *Teretrurus sanguineus* (FMNH 265872). (**E**) *M. tener* (FMNH 36758). (**F**) *M. tener* (FMNH 36753). De, ductuli efferentes; Ep, ductus epididymis; Rt, rete testis; Se, serous membrane; Sp, sperm; St, seminiferous tubules; Vp, visceral pleuroperitoneum.

testis of only one species, *P. platurus*, in which spermiation is evident in the testes. This indicates that sperm do not reside long in the rete testis and quickly pass into the ductuli efferentes.

The epithelium of the rete testis varies from simple squamous to simple cuboidal and is nonciliated. Variation occurs in the number of rete testis tubules, ranging from two in *A. eydouxii* and

L. colubrina to 19 in *H. lamberti*. The sample size for each species included in this study is small, but body and testicular length do not seem related to the number of rete testes tubules. Both *L. colubrina* and *H. lamberti* were among the longest snakes, with SVL:testicular lengths of 92.5 cm:2.29 cm and 80.3 cm:1.63 cm, respectively, in specimens in which rete testes were counted. One of the smallest snakes examined, a *L. dulcis* with SVL:testicular length of 11.2 cm:0.68 cm had 12 rete tubules. In specimens with numerous rete tubules, branching occurs from seminiferous tubules along the entire length of the testis. The midsagittal section through the testis and proximal ducts of *E. greyi* illustrated in Figure 1A shows rete testes in at least three different locations along the length of the testis.

Tubules of the distal rete testis branch into the ductuli efferentes. Because of branching and convolutions, the ductuli efferentes appear as the most numerous ducts within the epididymal sheath (Figs. 1–3). Transitions from the rete tubules to the ductuli efferentes occur in the epididymal sheath, such that all of the latter ducts are entirely extra-testicular within the epididymal sheath. The ductuli efferentes are characterized by a ciliated, cuboidal epithelium.

The ductus epididymis is the widest duct and is slightly folded so that a sagittal section will show several portions (Fig. 1A), but usually only one portion occurs in a transverse section (Figs. 1B, 2, and 3). At the light microscopy level, the epithelium of the ductus epididymis is nonciliated and pseudostratified with columnar principal cells and scattered basal cells. No evidence for regionalization of the ductus epididymis was found in any species.

The fact that the testis of *L. dulcis* is lobed is well known (Fox, 1965), but we found a heretofore unreported segmentation of the testis of *M. tener*. A double layer of serous membrane derived from the visceral pleuroperitoneum of anterior and posterior portions divides the testis transversely into halves (Fig. 3F).

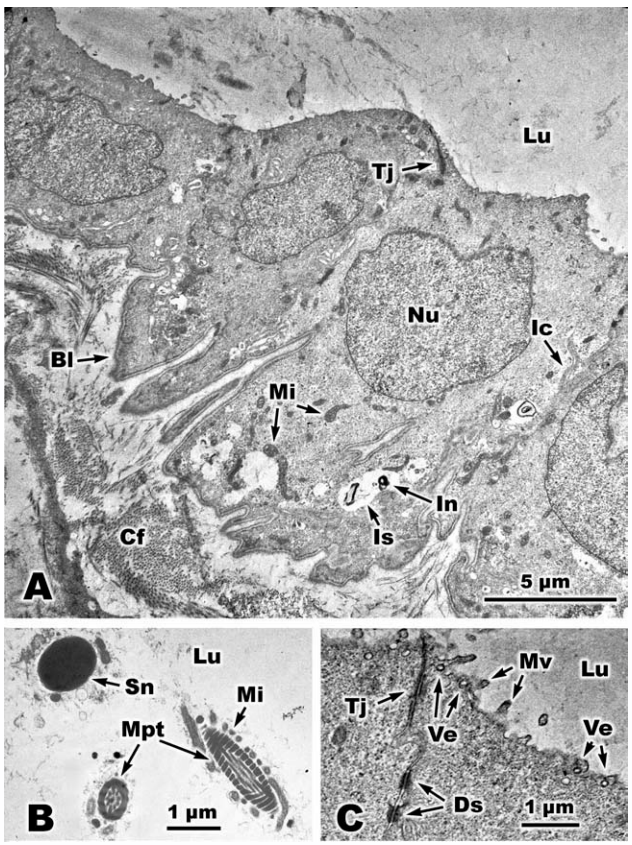

Fig. 4. TEM of the rete testis of *P. platurus* (SLU 0133). (**A**) Overview of epithelial cells. (**B**) Degenerating sperm cell. (**C**) Luminal border with numerous vesicles. Bl, basal lamina; Cf, collagen fibers; Ds, desmosomes; Ic, intercellular canaliculi; In, inclusion; Is, intercellular space; Lu, lumen; Mi, mitochondria; Mpt, midpiece of the tail; Mv, microvilli; Nu, nucleus; Sn, sperm nucleus; Tj, tight junction; Ve, vesicle.

Ultrastructure in *P. platurus*

Rete testis. The simple epithelium comprising the rete testis varies from squamous to low columnar and possesses nonciliated cells with basal borders indented to half the height of the cells (Fig. 4). Bundles of collagen fibers are scattered throughout the tunica propria, and smooth muscle is absent. The epithelium possesses large, irregularly shaped, often indented, euchromatic nuclei. Cells of the rete testis are separated by generally narrow intercellular canaliculi. Some sperm in the lumen appear to be undergoing degeneration, as the nuclear membrane is absent and the mitochondrial sheath around the midpiece of the tail is disrupted (Fig. 4B). The luminal border possesses short microvilli, between which small vesicles are scattered. Many of these vesicles appear with electron dense membranes that indicate they are coated (Fig. 4C). The only conspicuous organelles within the cells of the rete testis are small mitochondria that are scattered throughout the cytoplasm (Figs. 4A and 5).

The intercellular canaliculi in the apical portion of the cells have tight junctions at the luminal border followed by one or more desmosomes. More basally, the intercellular canaliculi often remain narrow and labyrinthine and lack additional junctional complexes (Figs. 4C and 5A,B). In some instances, however, cytoplasmic spaces that lack membranes merge with intercellular canaliculi (Fig. 5B). Once united with the intercellular canaliculi, the spaces acquire membranes. These intercellular spaces increase in size basally, and some contain electron dense inclusions that may represent cellular debris (Fig. 5A,D). Clusters of small vesicles are also found along the basal lamina (Fig. 5C). The basal ends of the intercellular canaliculi open directly into the tunica propria (Fig. 5D).

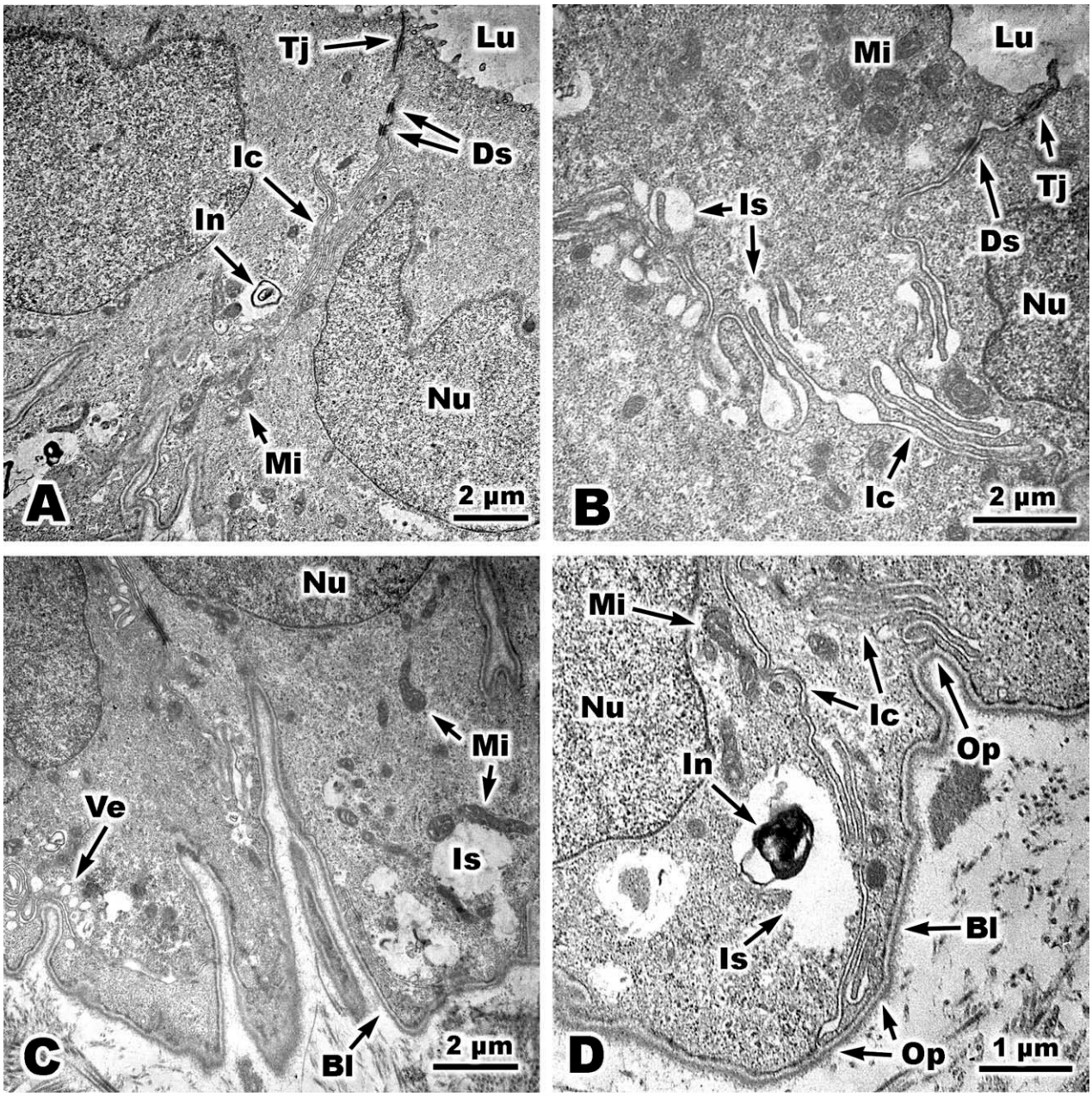

Fig. 5. TEM of the intercellular canaliculi and basal lamina of the rete testis of *P. platurus*. (**A**) Intercellular canaliculi with tight junctions and desmosomes. (**B**) Merging of intercellular spaces with narrow, labyrinthine intercellular canaliculi. (**C**) Small clusters of vesicles along the basal lamina. (**D**) Electron dense inclusion in the intercellular space and openings of the intercellular canaliculi into the tunica propria. Bl, basal lamina; De, desmosomes; En, endosome; Ic, intercellular canaliculi; In, inclusion; Is, intercellular space; Lu, lumen; Mi, mitochondria; Nu, nucleus; Op, opening; Tj, tight junction; Ve, vesicle.

Ductuli efferentes. The simple columnar epithelium of the ductuli efferentes possesses ciliated and nonciliated cells with a well-developed brush border of microvilli (Figs. 6 and 7A,B). Round, basal, and euchromatic nuclei are visible and a central, dense nucleolus is prominent in some sections (Fig. 6). As with the rete testis, the intercellular canaliculi possess junctional complexes apically. Inferior to these, the canaliculi are less labyrinthine than in the rete testis, but intercellular spaces are again conspicuous, and are largest basally (Figs. 6 and 7A,C,D). The intercellular spaces again appear to arise from cytoplasmic spaces lacking membranes. Some of these intercellular spaces contain small dense inclusions (Fig. 7C), and all possess a flocculent material. Along the luminal border, however, numerous vesicles with electron dense membranes are found

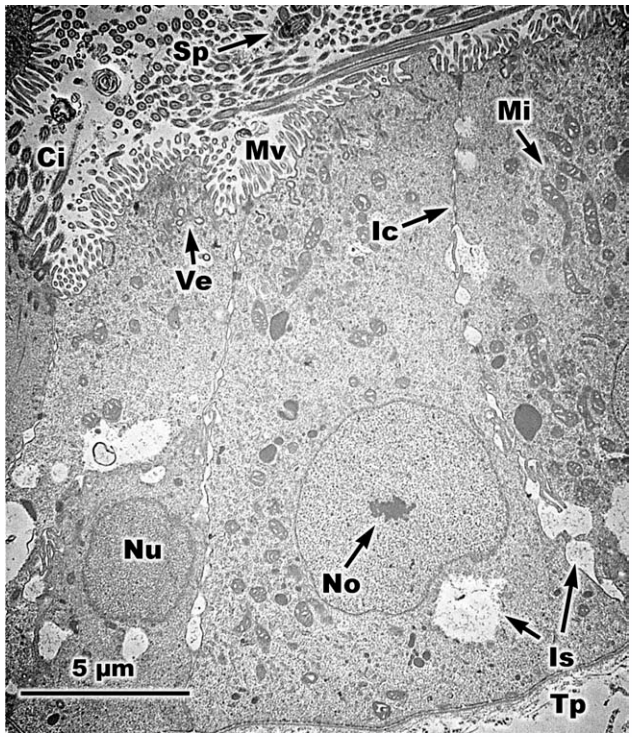

Fig. 6. TEM overview of the epithelial cells of the ductuli efferentes of *P. platurus*. Note ciliated and nonciliated cells, the presence of intercellular spaces, and membrane bound vesicles along the luminal border. Ci, cilia; Ic, intercellular canaliculi; Is, intercellular space; Mi, mitochondria; Mv, microvilli; No, nucleolus; Nu, nucleus; Sp, sperm; Tp, tunica propria; Ve, vesicles.

between microvilli of noncilated cells (Figs. 6 and 7A). Secretion by an apocrine process is indicated by apical blebs (Fig. 7B). Small membranous tubular structures are found along the luminal border (Fig. 7B) and deeper in the cytoplasm (Fig. 7C).

Sperm, more numerous in the lumen of the ductuli efferentes than in the rete testis, once again appear to be degenerating (Fig. 7A). Irregularly shaped electron-dense lysosomes are scattered throughout the cytoplasm. Small membranous vacuoles interpreted to be endosomes occur apically (Fig. 7B). As in the rete testis, the intercellular canaliculi have narrow openings into the surrounding connective tissue (Fig. 7D).

Ductus epididymis. The ductus epididymis is pseudostratified with three types of cells: apical, basal, and principal cells (Fig. 8). Each cell type occupies a particular region of the epithelium and has dense, euchromatic nuclei. Apical cells have oval nuclei along the luminal border, and their cytoplasm does not extend to the basal lamina. Basal cells with flattened nuclei and scant cytoplasm occur sporadically just inside the basal lamina. Principal cells are columnar and extend from the basal lamina to the lumen, with the round nucleus in the lower third of the cell.

Several layers of smooth muscle are present around the ductus epididymis (Fig. 8); smooth muscle is absent around the rete testis and ductuli efferentes. The most notable feature of the ductus epididymis, however, is the extensive, expanded intercellular spaces that in some cells occupy the majority of the intracellular area (Fig. 8).

Apical cells are marked by numerous small mitochondria, many of which abut the luminal membrane, and possess small microvilli (Fig. 9A). The intercellular canaliculi have tight junctions at the luminal end and, as in more proximal ducts, narrow openings into the basal lamina (Fig. 9B). Occasionally, the intercellular canaliculi have a desmosome in narrow areas between intercellular spaces. Basal cells appear to lack cytoplasmic organelles (Fig. 10A).

Some principal cells possess small biphasic secretory vacuoles along their luminal border (Fig. 8B). These vacuoles are biphasic with a moderately dense core surrounded by a ring of less dense material. The principal cells have scattered microvilli, and occasionally a single cilium occurs (Fig. 10B). Small Golgi vesicles occur in supranuclear areas, but no condensing vacuoles are elaborated (Fig. 10C). Rough endoplasmic reticulum (Rer), however, is abundant in the cytoplasm between enlarged intercellular spaces inferior to the apical cell layer (Figs. 9B and 10D). The Rer consists of tightly packed, large, sparsely granulated dense cisternae.

DISCUSSION

General Organization of the Testicular Ducts

The general organization of the testicular ducts of the species examined is concordant with the recent descriptions by Siegel et al. (2009) for *A. piscivorus* and Sever (2010) for *S. pygaea*, and species included in the review by Trauth and Sever (2011). We found no evidence for regionalization of the ductus epididymis, despite descriptions of up to four regions in agamid and lacertid lizards corresponding to the initial segment, caput, corpus, and cauda of mammals (Desantis et al., 2002; Akbarsha et al., 2006a, 2006b, 2007).

The number of rete tubules varies among the snakes used in this study and among squamate species described in previous studies. Alverdes (1928) reported an extraordinary range of rete tubules from 3 to 33 in *Natrix natrix*. Fox (1952) and Volsøe (1944) did not provide counts of the number of rete tubules in *Thamnophis elegans* and *Vipera berus*, respectively, but described them as being numerous. Sever (2010) reported 5–7 rete tubules in *S. pygaea*. Various studies suggest that there is a general reduction in the number of rete tubules within lizards; only one rete tubule was reported in *Sitana ponticeriana* (Akbarsha et al., 2007), *Sceloporus undulatus* (Forbes, 1941), *Lactera* sp. (Martin

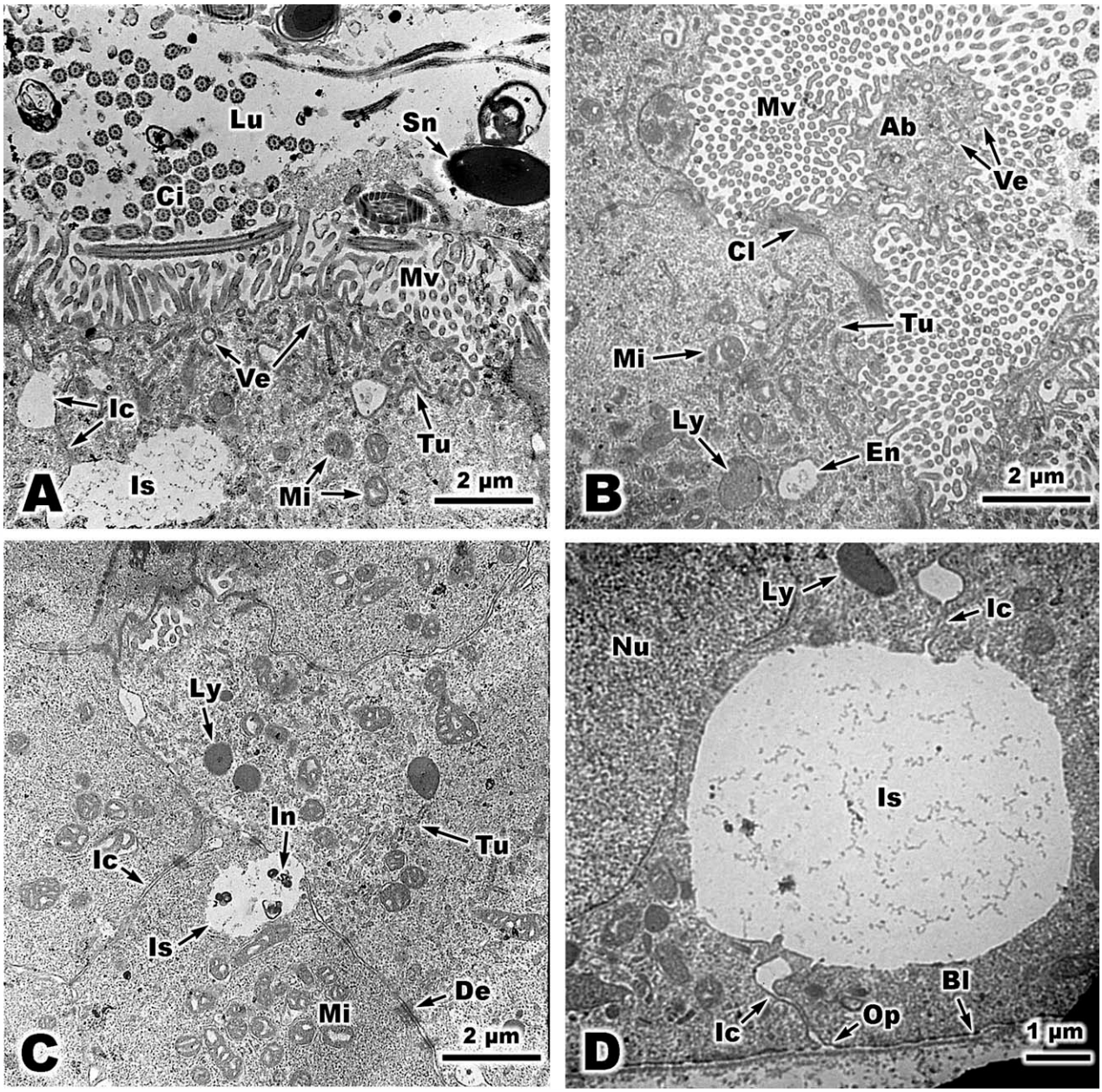

Fig. 7. TEM of the cytoplasm and luminal border of the ductuli efferentes of *P. platurus*. (**A**) Occurrence of a vacuole and numerous membrane bound small vesicles apically and degenerating sperm in the lumen of a nonciliated cell. (**B**) Secretion by apocrine process as indicated by the presence of an apocrine bleb. (**C**) Presence of lysosomes, vacuoles, and tubular structures deeper in the cytoplasm. (**D**) Occurrence of large vacuole basally and opening of the intercellular canaliculi into the surrounding connective tissue. Ab, apocrine bleb; Bl, basal lamina; Ci, cilia; Cl, cleavage line; De, desmosomes; En, endosome; Ic, intercellular canaliculi; In, inclusion ; Lu, lumen; Ly, lysosome; Mi, mitochondria; Mv, microvilli; No, nucleolus; Nu, nucleus; Op, opening; Sn, sperm nucleus; Sp, sperm; Tu, tubules; Tp, tunica propria; Va, vacuole; Ve, vesicle.

Saint-Ange, 1854; Alverdes, 1926; Van den Broek, 1933), and *Hemidactylus turcicus* (Rheubert et al., 2010). Badir (1958) counted nine and six rete tubules in the scincid lizards *Chalcides ocellatus* and *Scincus scincus*, respectively. Volsøe (1944) proposed that snakes, with numerous rete tubules, represent the ancestral condition for the squamate rete testis, and that lizards, with one or few ducts, display the derived condition. It is interesting to note that *L. dulcis*, considered a basal snake, was found to possess numerous rete tubules (12).

Ultrastructure

Histochemical tests indicate a neutral carbohydrate and protein (although this reaction was

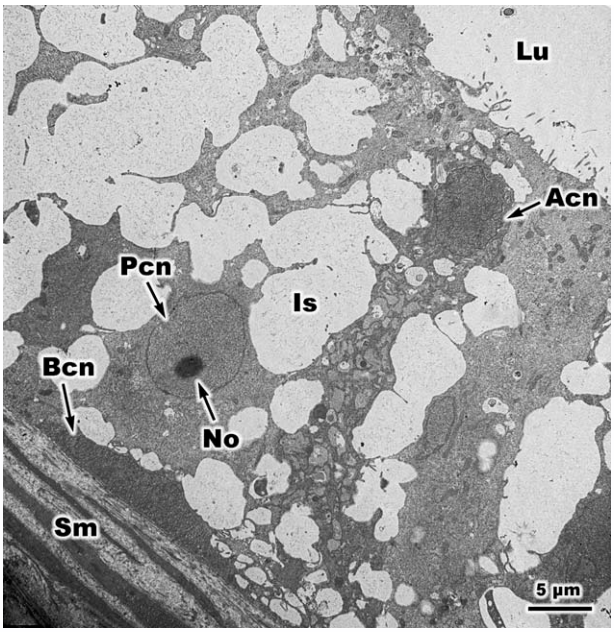

Fig. 8. TEM overview of the ductus epididymis of *P. platurus*. Note apical, basal, and principle cells and extensive intercellular spaces. Acn, apical cell nucleus; Bcn, basal cell nucleus; Is, intercellular space; Lu, lumen; No, nucleolus; Pcn, principle cell nucleus; Sm, smooth muscle.

weak) secretion from all regions of the anterior testicular ducts of *P. platurus*, so the challenge with ultrastructure is to determine the origin of these products. Transmission electron microscopy, however, reveals that secretory vacuoles are lacking in the rete testis and ductuli efferentes, so secretion in these ducts likely occurs by a constitutive pathway transporting products to the lumen by small vesicles as described previously in two snakes, *S. pygaea* (Sever, 2010) and *A. piscivorus* (Trauth and Sever, 2011) and in the proximal efferent ducts of mammals (Hoffer et al., 1973). Apical blebs in the ductuli efferentes of *P. platurus* often have numerous vesicles. Apical blebs in mammals upon fragmentation in the lumen potentially release glycoconjugates and enzymes, perhaps involved in sperm maturation (Hermo and Robaire, 2002; Hess, 2002).

Many of the vesicles found between microvilli have electron dense membranes characteristic of coated vesicles, and are likely associated with receptor-mediated endocytosis. The resorption of proteins, water, and ions in efferent ducts of mammals is known to depend on an elaborate system of microvilli, coated pits, apical tubules, endosomes, and lysosomes (Hess, 2002), and all of those structures and organelles are found in the cytoplasm of the ductuli efferentes of *P. platurus*.

One of the most conspicuous features of the proximal efferent ducts of *P. platurus* is intracytoplasmic cavities that lack membranes and the widened intercellular canaliculi, especially basally in the ductuli efferentes and between principal cells in the ductus epididymis. This cytology is very similar to the mammalian ductus deferens as described by Hermo et al. (2002). They suggested

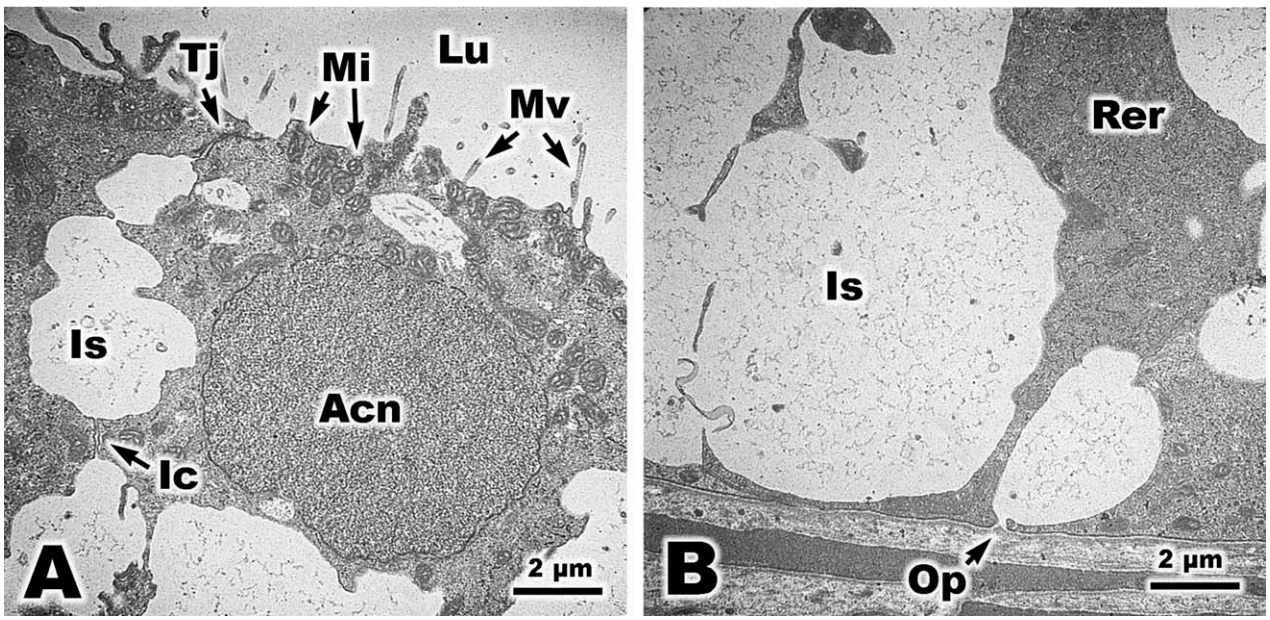

Fig. 9. TEM of apical cells and basal border of the ductus epididymis of *P. platurus*. Note large intercellular spaces. (**A**) Apical cell with euchromatic nuclei, small microvilli, and numerous small mitochondria. (**B**) Openings of intercellular canaliculi into the basal lamina and presence of rough endoplasmic reticulum between intercellular spaces. Acn, apical cell nucleus; Ic, intercellular canaliculi; Is, intercellular space; Lu, lumen; Mi, mitochondria; Mv, microvilli; Op, opening; Rer, rough endoplasmic reticulum; Tj, tight junction.

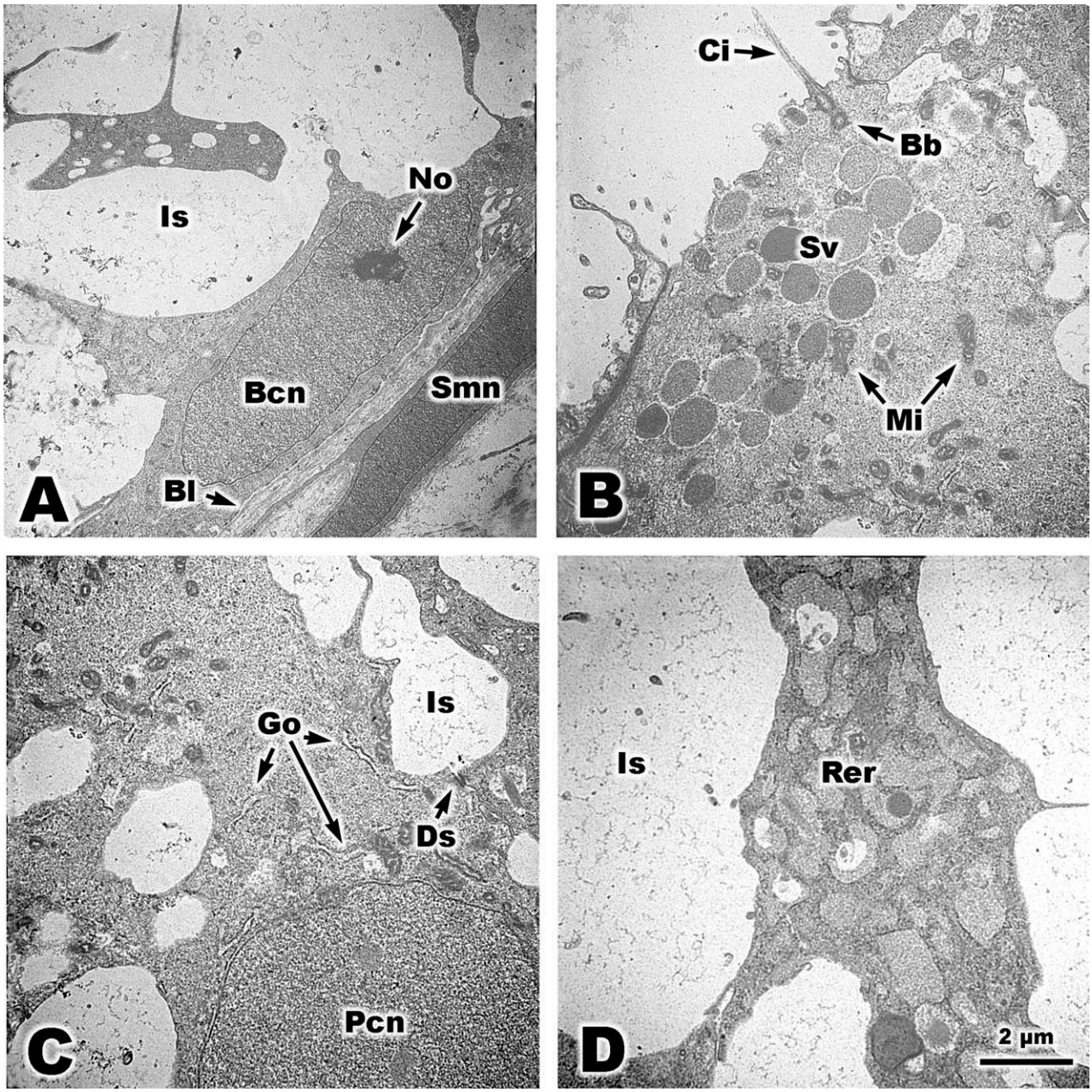

Fig. 10. TEM of the ductus epididymis of *P. platurus*. (**A**) Basal cell lacking cytoplasmic organelles and adjacent large intercellular spaces. (**B**) Principle cells with secretory vacuoles and a single cilium. (**C**) Principle cells containing a Golgi complex and intercellular canaliculi with large intercellular spaces and a desmosome. (**D**) Principle cell dominated by intercellular spaces and an abundance of rough endoplasmic reticulum. Bcn, basal cell nucleus; Bl, basal lamina; Bb, basal bodies; Ci, cilia; Ds, desmosomes; Go, Golgi complex; Is, intercellular space; Mi, mitochondria; No, nucleolus; Pcn, principle cell nucleus; Rer, rough endoplasmic reticulum; Smn, smooth muscle nucleus; Sv, secretory vacuoles.

that the intracytoplasmic spaces and dilated spaces between adjacent cells are areas of water accumulation and transport. In *P. platurus*, tight junctions seal the apical border but few junctional complexes occur elsewhere, and the narrowed intercellular canaliculi open freely into the surrounding stroma in all regions. 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). We are unaware of any studies on AQPs in the male reproductive tracts of reptiles, so this is an area ripe for investigation.

The ductus epididymides of squamates studied by both light and electron microscopy vary considerably in how secretory products are packaged. Lizards may have numerous large, electron dense

secretory vacuoles, e.g., *Lacerta vivipara* (Mesure et al., 1991) and *S. ponticeriana* (Akbarsha et al., 2006b), whereas others have smaller vacuoles or simply dense and heterogeneous cytoplasm (Dufaure and Saint Girons, 1984). Dufaure and Saint Girons (1984) indicated that snakes lack secretory activity in the epididymis, but ultrastructural work on *A. piscivorus* (Siegel et al., 2009) and *S. pygaea* (Sever, 2010) revealed constitutive secretory processes in those species. *P. platurus* is the first snake in which secretory vacuoles have been found in the apical cytoplasm of the principal cells. The biphasic appearance is characteristic of an inner dense protein core and an outer more lucent polysaccharide or glycoprotein (Depeiges and Dufaure, 1980). Spermiation was in progress in these snakes, and the ductus epididymis was probably not as hypertrophied as it would become later in the breeding season. Future work is necessary to determine the seasonal cycle of secretory activity.

P. platurus is unique among the few snakes studied so far in possession of apical cells, which are well known in mammals (Hermo and Robaire, 2002) and have also been reported in two lizards, *Calotes versicolor* (Meeran et al., 2001) and *Sitana ponticerana* (Akbarsha et al., 2006b). In both lizards, however, the apical cells were reported to have a narrow attachment to the basal lamina, whereas the apical cells in *P. platurus* are similar to mammals in being limited to the luminal border of the duct. Apical cells are characterized by numerous mitochondria. The function of apical cells is unclear, but in mammals they are known to endocytose substances from the lumen (Hermo and Robaire, 2002).

Finally, *P. platurus* is unique among snakes and other squamates in possessing a single cilium on scattered principal cells in the ductus epididymis. This condition has also been described in some birds (Aire, 2007).

Evolutionary Implications

Sever (2010) identified potential phylogenetic characters derived from ultrastructural studies of the amniotic reproductive tract (Table 1 in Sever, 2010), and Trauth and Sever (2011) called for descriptions of cytological variation and secretory activity within a broad range of squamate taxa. This study contributes to this database by reporting conserved testicular duct histology among snakes from basal to more derived clades. When ultrastructural observations are included, however, some novel cytological characters for squamates were found for the testicular ducts, especially the ductus epididymis, of *P. platurus*. Thus, variation is missed in studies limited to gross morphology and light microscopy, and future studies should strive to examine ultrastructure to uncover the details of cytological complexity.

ACKNOWLEDGMENTS

The authors thank Harold Voris and Alan Resetar of the Field Museum and Eric Smith and Thomas Eimermacher of University of Texas Arlington for the loan of specimens. Assistance with permits to conduct fieldwork in Costa Rica was aided by Francisco Campos from the Organization for Tropical Studies. The authors thank Mindy Mayon and Melania Garcia for help in collecting *Pelamis* and Thomas Beach for help in tissue preparation. Thanks to Justin Rheubert for critically reading the manuscript.

LITERATURE CITED

Aire TA. 2007. Anatomy of the testis and male reproductive tract. In: Jamieson BBG, editor. Reproductive Biology and Phylogeny of Birds. Enfield, NH: Science Publishers. pp. 37–113.

Akbarsha MA, Kadalmani B, Tamilarasan V. 2006a. Histological variation along and ultrastructural organization of the epithelium of the ductus epididymides of the fan-throated lizard *Sitana ponticeriana* Cuvier. Acta Zool (Stockholm) 87:181–196.

Akbarsha MA, Tamilarasan V, Kadalmani B. 2006b. Light and electron microscopic observations of fabrication, release, and fate of biphasic secretion granules produced by epididymal epithelial principal cell of the fan-throated lizard *Sitana ponticeriana* Cuvier. J Morphol 267:713–729.

Akbarsha MA, Kadalmani B, Tamilarasan V. 2007. Efferent ductules of the fan-throated lizard *Sitana ponticeriana* Cuvier. J Morphol 267:713–729.

Alverdes K. 1926. Die samenableitungswege der eidechsen. Z fur Mikrosk Anat Forsch 6:420–442.

Alverdes K. 1928. Die Epididymis der Sauropsiden im Vergleich zu Säugetier und Mensch. Z Mikrosk Anat Forsch 1928:405–471

Badir N. 1958. Seasonal variation of the male urogenital organs of *Scincus scincus* L. and *Chalcides ocellatus* Forsk. Zeit Wissen Zool 160:290–351.

Badran HM, Hermo LS. 2002. Expresion and regulation of aquaporins 1, 8, and 9 in the testis, efferent ducts, and epididymis of adult rats and during postnatal development. J Androl 23:358–373.

Burbrink FT, Crother BI. 2011. Evolution and taxonomy of snakes. In: Aldridge RD, Sever DM, editors. Reproductive Biology and Phylogeny of Snakes. Boca Raton, FL: CRC Press. pp 19–53.

Burns GW. 1984. Aspects of population movements and reproductive biology of *Aipysurus laevis*, the Olive Sea Snake. PhD Dissertation, University of New England, Armidale, NSW.

Cadle JE, Gorman GC. 1981. Albumin immunological evidence and the relationships of sea snakes. J Herpetol 15:329–334.

Depeiges A, Dufaure JP. 1980. Major proteins secreted by the epididymis of *Lacerta vivipara*. Isolation and characterization by electrophoresis of the central core. Biochim Biophys Acta 628:109–115.

Desantis S, Labate M, Labate GM, Cirillo F. 2002. Evidence of regional differences in the lectin histochemistry along the ductus epididymis of the lizard, *Podarcis sicula* Raf. Histochem J 34:123–130.

Dufaure J, Saint Girons H. 1984. Histolgie compare de l'epididyme et de ses secretions chez les reptiles (lezards et serpents). Arch d'Anat Micros 73:15–26.

Forbes TR. 1941. Observations on the urogenital anatomy of the adult male lizard, *Sceloporus*, and on the action of implanted pellets of testosterone and of estrone. J Morphol 68:31–69.

Fox W. 1952. Seasonal variation in the male reproductive system of Pacific coast garter snakes. J Morphol 90:481–553.

Fox W. 1965. A comparison of the male urogenital systems of blind snakes, Leptotyphlopidae and Typhlopidae. Herpetologica 21:241–256.

Gopalakrishnakone P, Kochva E. 1990. Venom glands and some associated muscles in sea snakes. J Morphol 205:85–96.

Gorman GC, Licht P, McCollum F. 1981. Annual reproductive patterns in three species of marine snakes from Central Phillippines. J Herpetol 15:335–354.

Guinea ML. 1981. The sea snakes of Fiji. Proceedings from the Fourth International Coral Reef Symposium, Manila, Philippines 2:581–585.

Guinea ML. 1986. Aspects of the biology of the common Fijian sea snake *Laticauda colubrina* (Schneider). MSc Thesis, University of the South Pacific, Suva, Fiji.

Hayat MA. 1993. Stains and Cytochemical Methods. New York: Plenum Press. p 455.

Heatwole H. 1999. Sea Snakes. Malabar, FL: Krieger. p 148.

Hermo L, Robaire B. 2002. Epididymal cell types and their functions. In: Robaire B and Hinton BT, editors. The Epididymis: From Molecules to Clinical Practice NY: Kluwer Academic/Plenum Publishers. pp 81–102.

Hermo L, Badran H, Andonian S. 2002. Structural organization and functions of the epithelium of the vas deferens. In: Robaire B and Hinton BT, editors. The Epididymis: From Molecules to Clinical Practice. NY: Kluwer Academic/Plenum Publishers. pp 233–250.

Hess RA. 2002. The efferent ductules: Structure and functions. In: Robaire B, Hinton BT, editors. The Epididymis: From Molecules to Clinical Practice. NY: Kluwer Academic/Plenum Publishers. pp 49–196.

Hoffer AP, Hamilton DW, Fawcett DW. 1973. The ultrastructurwe of the principal cells and intraepithelial leukocytes in the initial segment of the rat epididymis. Anat Rec 175:169–202.

Huang H-F, He R-H, Sun C-C, Zhang Y, Meng Q-X, Ma Y-Y. 2006. Function of aquaporins in female and male reproductive systems. Hum Reprod Update 12:785–789.

Keogh JS. 1998. Molecular phylogeny of elapid snakes and a consideration of their biogeographic history. Biol J Linn Soc 63:177–203.

Keogh JS, Shine R, Donnellan S. 1998. Phylogenetic relationships of terrestrial Australo-Papuan elapid snakes (Subfamily Hydrophiine) based on cytochrome b and 16S rRNA sequences. Mol Phylogenet Evol 10:67–81.

Lemen CA, Voris HK. 1981. A comparison of reproductive strategies among marine snakes. J Anim Ecol 50:89–101.

Lillywhite HB, Babonis LS, Sheeny CM, Ming-Chung T. 2008. Sea snakes (*Laticauda* spp.) require fresh drinking water: Implication for the distribution and persistence of populations. Physiol Biochem Zool 81:785–796.

Lukoschek V, Keogh JS. 2006. Molecular phylogeny of sea snakes reveals a rapidly diverged adaptive radiation. Biol J Linn Soc 89:523–539.

Martin Saint-Ange GJ. 1854. Étude de l'appareil reproducteur dan les cinq d'animaux vertébrés, au point de vue anatomique, physiologique et zoologique. Paris, France: Imprimeri Impériale. pp 281.

Meeran MM, Daisy P, Akbarsha MA. 2001. Histological differentiation along the ductus epididymis of the lizard *Calotes versicolor* Daudin. J Anim Morpol Physiol 48:85–96.

Mesure M, Chevalier M, Depeiges A, Faure J, Dufaure JP. 1991. Structure and ultrastructure of the epididymis of the viviparous lizard during the annual hormonal cycle: Changes of the epithelium related to secretory activity. J Morphol 210:133–145.

Minton SA. 1975. Geographic distribution of sea snakes. In: Dunson WA, editor. The Biology of Sea Snakes. Baltimore, MD: University Park Press. pp 21–32.

Morgera A. 1905. La relazione tra it testicolo ed il deferente di alcuni Rettili. Boll Soc Nat Napoli 18:114–128.

Rheubert JL, Sever DM, Geheber AD, Siegel DS. 2010. Proximal testicular ducts of the Mediterranean Gecko (*Hemidactylus turcicus*). Anat Rec 293:2176–2192.

Scanlon JD, Lee MSY. 2011. The major clades of living snakes: Morphological evolution, molecular phylogeny, and divergence dates. In: Aldridge RD, Sever DM, editors. Reproductive Biology and Phylogeny of Snakes. Boca Raton, FL: CRC Press. pp 55–95.

Sever DM. 2010. Ultrastructure of the reproductive system of the black swamp snake (*Seminatrix pygaea*). VI. Anterior testicular ducts and their nomenclature. J Morphol 271: 104–115.

Siegel DS, Sever DM, Rheubert JL, Gribbins KM. 2009. Reproductive biology of *Agkistrodon piscivorus* Lacépède (Squamata, Serpentes, Viperidae, Crotalinae). Herpetol Monogr 23:74–107.

Slowinksi JB, Knight A, Rooney AP. 1997. Inferring species trees from gene trees: a phylogenetic analysis of Elapidae (Serpentes) based on the amino acid sequenes of venom proteins. Mol Phylgenet Evol 8:349–362.

Trauth SE, Sever DM. 2011. Male urogenital ducts and cloacal anatomy. In: Aldridge RD, Sever DM, editors. Reproductive Biology and Phylogeny of Snakes. Boca Raton, FL: CRC Press. pp 411–475.

Van den Broek AJP. 1933. Gonaden und susführungsgänge. In: Bolk L, Göpper E, Kallius E, Lubosh W, editors. Vergleichenden Anatomie der Wirbelthiere, Vol. 6. Berlin: Urban and Schwarzenberg. pp 1–154.

Volsøe H. 1944. Structure and seasonal variation of the male reproductive organs of *Vipera berus* (L.). Spolia Zool Mus Haun 5:1–157.

Voris HK. 1977. A phylogeny of sea snakes (Hydrophiidae). Fieldiana Zool 70:79–166.

Voris HK, Jayne BC. 1979. Growth, reproduction, and population structure of a marine snake, *Enhydrina schistose* (Hydrophiidae). Copeia 1979:307–318.

Zaniboni L, Akuffo V, Baskt MR. 2004. Aquaporins are observed in the duct epithelia of the epididymal region of the large white turkey. Poult Sci 83:1917–1920.