Ultrastructure of the Reproductive System of the Black Swamp Snake (*Seminatrix pygaea*). VI. Anterior Testicular Ducts and Their Nomenclature

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ABSTRACT In this study, the anterior testicular ducts of the North American natricine snake *Seminatrix pygaea* are described using light and electron microscopy. From the seminiferous tubules, the rete testis passes into the epididymal sheath, a structure along the medial border of the testis heavily invested with collagen fibers. The rete testis consists of simple, nonciliated cuboidal epithelium (principal cells). The intratesticular ducts of the rete testis are narrow (50–70 μm) at their junction with the seminiferous tubules, widen (80–100 μm) as they extend extratesticularly, and divide into smaller branches as they anastomose with the next tubules, the ductuli efferentes. The ductuli efferentes are lined by simple cuboidal epithelium but possess nonciliated principal cells as well as ciliated cells. These are the only ducts in the male reproductive system with ciliated cells. The ductuli efferentes are narrow (25–45 μm), divide into numerous branches, and are highly convoluted. The ductus epididymis is the largest duct in diameter (240–330 μm), and the diameter widens and the epithelium thins posteriorly. The ductus epididymis is lined by nonciliated, columnar principal cells and basal cells. No regional differences in the ductus epididymis are apparent. Ultrastructural evidence suggests that all of the nonciliated principal cells in each of the anterior testicular ducts function in both absorption and secretion. Absorption occurs via small endocytic vesicles, some of which appear coated. 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. The secretory product is a glycoprotein. Overall, the characteristics of the anterior testicular ducts of this snake are concordant with those of other amniotes, and the traditional names used for snakes are changed to conform with those used for other sauropsids and mammals. J. Morphol. 000:000–000, 2009.

KEY WORDS: Reptilia; Squamata; serpentes; *Seminatrix*; reproduction; epididymis; histology; ultrastructure

INTRODUCTION

This article represents the sixth in a series on the reproductive anatomy of *Seminatrix pygaea*, a natricine snake from the southeastern United States. Other articles in this series have described female sperm storage (Sever and Ryan, 1999), the oviducal cycle (Sever et al., 2000), renal sexual segment (Sever et al., 2002), the ductus deferens (Sever, 2004), and spermatogenesis (Gribbins et al., 2005). The current study is concerned with the anterior testicular ducts that pass sperm from the seminiferous tubules to the ductus deferens. These ducts are involved in reabsorption of luminal fluid, resulting in the concentration of sperm, and possess a secretory function that appears to vary considerably among various vertebrate groups (Hess, 2002).

Sperm formed in the seminiferous tubules of snakes and lizards (i.e., squamates) pass out of the testes into a series of ducts sequentially termed the ductuli efferentes, ductuli epididymides, ductus epididymis, and ductus deferens, which unites with the ureter in the anterior wall of the cloaca (Van Den Broek, 1933; Volsøe, 1944). The names of the most anterior ducts, the "ductuli efferentes" and "ductuli epididymides" were retained in the last review of the system by H. Fox (1977). The most recent detailed studies on these ducts in snakes, however, were by W. Fox (1952) and Saint Girons (1957) who simply adopted the terminology of Volsøe (1944).

Since the last studies on snakes, histological as well as ultrastructural studies have been done on lizards (Mesure et al., 1991; Desantis et al., 2002; Akbarsha et al., 2006a, 2006b, 2007), a turtle (Holmes and Gist, 2004), and a crocodilian (Guerrero et al., 2004), as well as numerous species of birds (reviewed by Aire, 2007) and mammals (reviewed in Robaire and Hinton, 2002). All of these studies have adopted a common nomenclature

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based on similarities in development, form, and function (Jones, 1998, 2002).

Jones (1998) homologized the squamate "ductuli efferentes" as defined by Volsøe (1944) with the mammalian extratesticular rete testis on developmental grounds, as both are derived from the rete blastema. On a similar basis, Jones (1998) considered the "ductuli epididymides" to be homologues of the mammalian ductuli efferentes because both are derived from mesonephric tubules. Akbarsha et al. (2007) followed the terminology of Jones (1998) in their study on the efferent ducts of the Asian lizard, *Sitana ponticeriana*.

In the current study, I present the first ultrastructural descriptions of the anterior testicular ducts of a snake, with a primary goal of determining whether they are unique among amniotes, and deserving a different nomenclature, or whether they appear structurally and functionally homologous to those ducts in other amniotes.

MATERIALS AND METHODS

Seminatrix pygaea is a small [20–40 cm snout-vent length (SVL) as adults], highly aquatic snake that is limited to the southern Atlantic coastal plain of the United States (Dorcas et al., 1998). All specimens used in this study were collected at Ellenton Bay, located on the Department of Energy's Savannah River Site in Aiken County, South Carolina. This "Carolina bay" is freshwater, 10 ha, shallow (2 m maximum depth), and relatively permanent (Gibbons and Semlitsch, 1991). The population of *S. pygaea* at this locale is the largest known for the species (Gibbons and Semlitsch, 1991).

Collections were made during four periods in 1998 (10 May, 7 June, 22–24 July, and 29 September–2 October), and one period in 1999 (17–22 March). Snakes were collected in unbaited minnow traps and from under cover-boards alongside the bay. Specimens were sacrificed within a week of capture. Two snakes were examined per collection, for a total sample of 10 snakes. Tissues from one snake each month were embedded in epoxy resin for transmission electron microscopy (TEM) and glycol methacrylate for light microscopy (LM), except no preparation for TEM was made from the July sample. Tissues from the other snake examined each month were prepared by the paraffin method for light microscopy. SVLs of the snakes ranged from 23.8 to 29.5 cm, and all were sexually mature.

Specimens were killed by a lethal injection (3–5 ml) of 10% sodium pentobarbital (Abbott Laboratories, North Chicago, IL) in 70% ethanol. This procedure was approved by the Animal Care and Use Committee of Saint Mary's College, Notre Dame, Indiana, where the injections were done. After death, SVL was measured from the tip of the snout to the posterior end of the cloacal orifice. Carcasses of all specimens were preserved in neutral-buffered formalin (NBF) and are deposited in the vertebrate collection of Southeastern Louisiana University.

For LM examination, tissues were initially fixed in NBF, rinsed in water, dehydrated in ethanol, cleared in toluene, and embedded in paraffin or glycol methacrylate (JB-4 Plus, Electron Microscopy Sciences, Port Washington, PA) plastic resin. Paraffin sections (10 μm) were cut with a rotary microtome and affixed to albuminized slides. Alternate paraffin slides from each specimen were stained with hematoxylin-eosin or methylene blue-eosin (general histology), bromphenol blue (BB, for proteins), and alcian blue 8GX at pH 2.5 (AB, for primarily carboxylated glycosaminoglycans) followed by the periodic acid-Schiff's procedure (PAS, for neutral carbohydrates and sialic acids). Sections (2 μm) from tissues embedded in JB4 were stained with methylene blue and basic fuschin. Procedures followed Dawes (1979), Humason (1979), and Kiernan (1990).

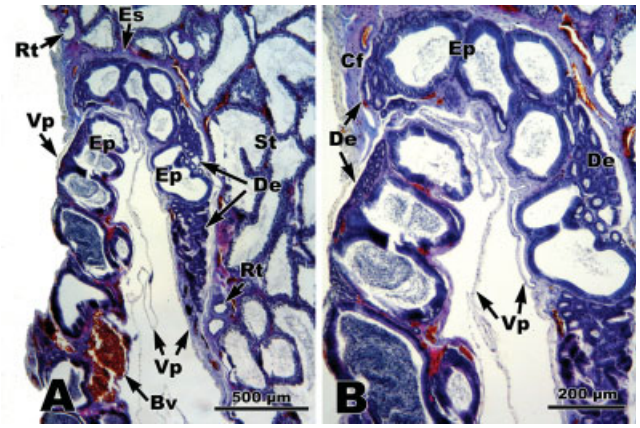

Fig. 1. Light micrographs of sagittal sections through the testis of *Seminatrix pygaea* collected in October and stained with methylene blue-eosin. **A**: Relationship of seminiferous tubules and rete testes to the ductuli efferentes and epididymis in the epididymal sheath. **B**: Presence of the ductuli efferentes in both the free (left) and attached (right) portions of the epididymal sheath. Bv, blood vessel; CF, collagen fibers; De, ductuli efferentes; Ep, ductus epididymis; Es, epididymal sheath; Rt, rete testis; St, seminiferous tubules; Vp, visceral pleuroperitoneum. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Tissue for TEM was trimmed into 1-mm blocks and fixed in a 1:1 solution of 2.5% glutaraldehyde in Millonig's phosphate buffer and 3.7% formaldehyde buffered to pH 7.2 with monobasic and dibasic phosphate. After initial fixation, tissues were rinsed in distilled, deionized water, postfixed in 2% osmium tetroxide, dehydrated through a graded series of ethanol, cleared in propylene oxide, and polymerized in an epoxy resin (Embed 812, Electron Microscopy Sciences, Port Washington, PA). Plastic sections were cut with an RMC MT7 ultramicrotome (Research and Manufacturing Co., Tucson, AZ). Semi-thin sections (500 nm) for LM were stained with toluidine blue, and ultrathin sections for TEM were placed on uncoated copper grids and stained with uranyl acetate and lead citrate. TEM observations were made with a Hitachi H-300 (Nissei Sangyo America, Mountain View, CA).

RESULTS

The testes do not have a dense tunica albuginea but rather a thin layer of collagen fibers border the seminiferous tubules, and superficial to the collagen layer is the visceral pleuroperitoneum. In the region of the excurrent ducts, the tunica propria of the serosa splits, becomes thickened and invested with numerous blood vessels and fibrous connective tissue; smooth muscle is not apparent. Distal ends of seminiferous tubules as well as the anterior testicular ducts and the adrenal gland are encased in this capsule, called by Siegel et al. (in press), the epididymal sheath. The epididymal sheath has a portion attached to the testis and a portion that lies free of the testis (Fig. 1).

From the seminiferous tubules, five to seven tubules representing the rete testis branch segmentally from anterior to posterior ends of the

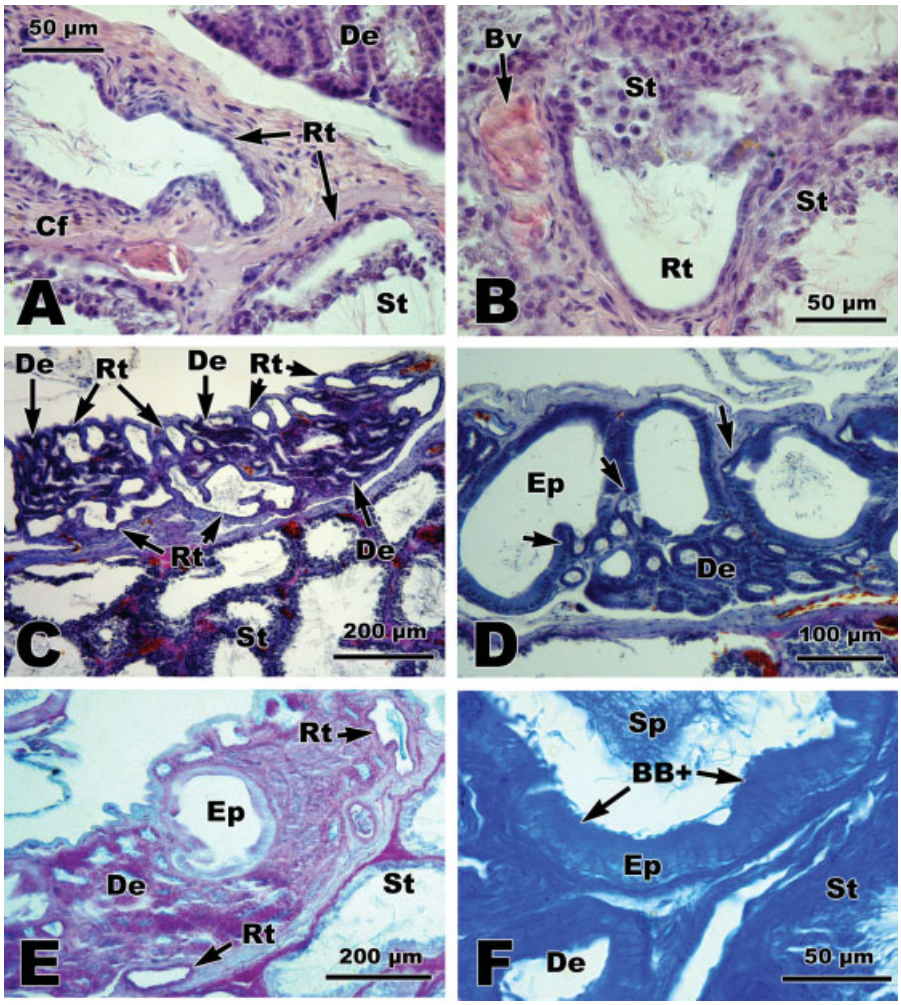

Fig. 2. Light micrographs through the anterior testicular ducts of *Seminatrix pygaea* collected in October. **A**, **B**: Intratesticular segments of the rete testis showing merger with seminiferous tubules, stained with hematoxylin-eosin. **C**: Extratesticular rete testis merging with ductuli efferentes in the attached epididymal sheath, stained with methylene blue-eosin. **D**: Ductuli efferentes merging (unlabeled arrows) with the ductus epididymis in the attached epididymal sheath, stained with methylene blue-eosin. **E**: PAS/AB reaction of anterior testicular ducts. Intensity of reddish stain reflects PAS+ reactions. **F**: BB reaction of anterior testicular ducts. Intensity of dark blue stain reflects BB+ reactions. Bv, blood vessel; Cf, collagen fibers; De, ductuli efferentes; Ep, ductus epididymis; Rt, rete testis; Sp, sperm; St, seminiferous tubules.

testes (see Fig. 1). The proximal ends connecting to the seminiferous tubules are narrower in diameter (50–70 µm) than the distal portions (80–100 µm; Fig. 2A,B). The proximal ends could be considered "intratesticular," but the point seems moot as even the distal ends of the seminiferous tubules extend into the epididymal sheath (Fig. 1A). The distal ends, however, certainly must be considered extratesticular as they divide and narrow at their junction with the next tubules, the ductuli efferentes, in the epididymal sheath (Fig. 2C). The rete testis is not highly convoluted but relatively straight. The epithelium of the rete testis varies from simple squamous to simple cuboidal (Fig. 2A,B).

Tubules of the distal rete testis branch into the ductuli efferentes, which are smaller in diameter (25–45 µm), quite convoluted, and appear to branch extensively (Figs. 1, 2C). The ductuli efferentes are entirely within the epididymal sheath. Most of the ductuli efferentes join with the initial segment of the epididymis, which is in the portion of the epididymal sheath that is attached to the testis (Fig. 1A, 2D).

The initial portion of the epididymis consists of six to eight loops that pass anteriorly, and then pass posteriorly in the free portion of the epididymal sheath along the posterior two-thirds of the testis (Fig. 1A). Some tubules of the ductuli efferentes continue into the most cranial end of the free portion of the epididymal sheath (Fig. 1B). The free portion of the epididymal sheath has numerous blood vessels. Although not visible in the section illustrated, the adrenal gland lies

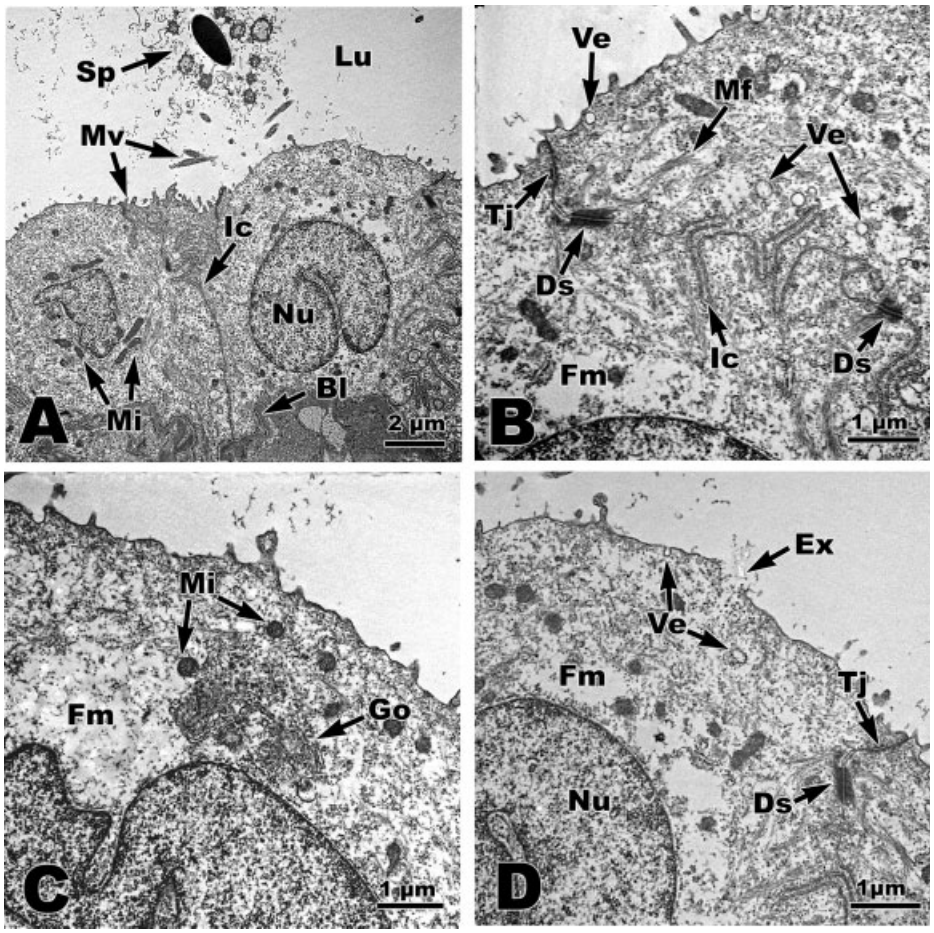

Fig. 3. TEM of the rete testis of *Seminatrix pygaea* collected in October. Note large, indented nuclei. **A**: Overview of epithelial cells. **B**: Apical cytoplasm showing small vesicles and labyrinthine, narrow intercellular canaliculi. **C**: Supranuclear flocculent material and Golgi complex. **D**: Possible exocytosis of materials (Ex) and endocytosis into vesicles. Bl, basal lamina; Ds, desmosomes; Ex, exocytosis; Fm, flocculent material; Go, Golgi complex; Ic, intercellular canaliculi; Lu, lumen; Mf, microfilaments; Mi, mitochondria; Mv, microvilli; Nu, nucleus; Sp, sperm; Tj, tight junction; Ve, vesicle.

adjacent to the ductus epididymis in the free portion of the epididymal sheath.

The ductus epididymis varies greatly in diameter with measurements from 240 to 330 μm in various specimens. The diameter increases posteriorly and the epithelium becomes thinner. The ductus epididymis is tightly looped. The ductus epididymis becomes the ductus deferens at the posterior end of the testes, although at this point, no other defining difference occurs.

All portions of the anterior testicular ducts have basophilic cytoplasm that stains PAS+ for neutral carbohydrates and BB+ for proteins (Fig. 2E,F). The PAS+ reaction for the ductus epididymis, however, is lighter and more diffuse than for the other ducts. Little seasonal variation occurs except that the staining reactions for carbohydrates and proteins were less intense in June and July. Sperm occur in the ductus epididymis the entire year, but are found in the rete testis and ductuli efferentes only in the October sample, when spermiation occurs (Gribbins et al., 2005).

Ultrastructure

The basis for the ultrastructure descriptions will be the October sample, because sperm occur in all of the ducts at this time. The cuboidal epithelial cells of the rete testis have large, indented euchromatic nuclei without condensed nucleoli, and cells are separated by narrow intercellular canaliculi which are especially labrinythine apically (Fig. 3A,B). Short microvilli are present but not abundant (Fig. 3A). Electron-dense, elongate mitochondria are most numerous basally (Fig. 3A). At the luminal end of the intercellular canaliculi are tight junctions, and desmosomes occur irregularly along the length of the membranes (Fig. 3B). Numerous small vesicles occur along the luminal border and in the apical cytoplasm along with bundles of microfilaments (Fig. 3B). A flocculent material occurs in the supranuclear area of most epithelial cells, and Golgi complexes are observed, often in conjunction with spherical, electron-dense mitochondria (Fig. 3C). Although some vesicles

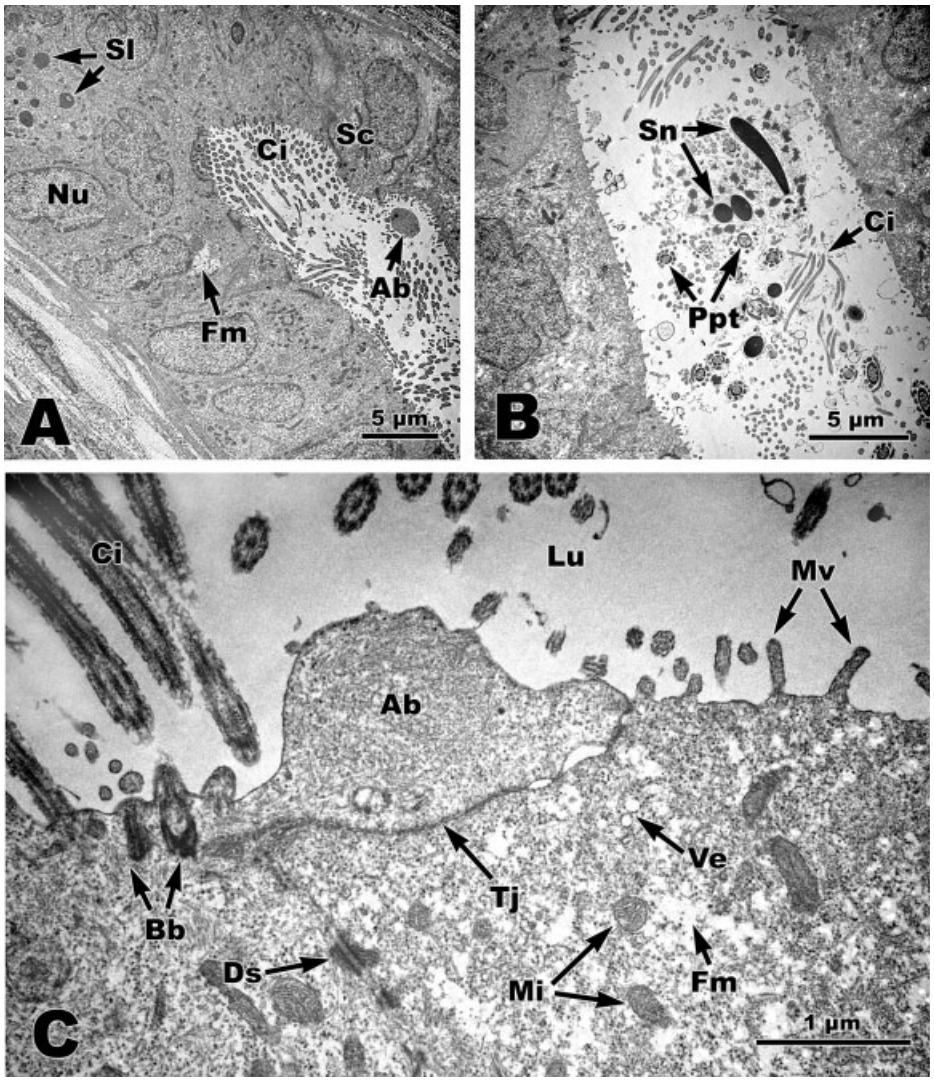

Fig. 4. TEM of the ductuli efferentes of *Seminatrix pygaea* collected in October. **A**: Overview of ciliated and non-ciliated cells. **B**: Luminal contents. **C**: Apical cytoplasm. Ab, apocrine bleb; Bb, basal bodies; Ci, cilia; Ds, desmosomes; Fm, flocculent material; Lu, lumen; Mi, mitochondria; Nu, nucleus; Ppt, principal piece of the tail; Sc, secretory cell; Sl, secondary lysosomes; Sn, sperm nucleus; Tj, tight junction; Ve, vesicles.

may be involved in absorption, evidence exists that exocytosis is also occurring by an apocrine process (Fig. 3D). The only seasonal variation noted was that the chromatin material becomes more condensed in spring and summer.

The ductuli efferentes have both ciliated and secretory cells (Fig. 4A). Because the lumina are narrow, the cilia seem to fill the space intermixed with sperm (Fig. 4B). Cytological features for both secretion and absorption are present. The apocrine secretion is much more abundant than in the rete testis, forming blebs that cleave off into the lumen (Fig. 4A,C). The supranuclear flocculent material is conspicuous in many secretory cells (Fig. 4A,C). Intercellular canaliculi are still narrow and have apical tight junctions followed by desmosomes deeper along the membranes (Fig. 4C).

At the base of microvilli, coated pits form larger vesicles, endosomes (Fig. 5A). Electron-dense lysosomes occur in the supranuclear regions, and the rough endoplasmic reticulum (Rer) is well developed (Fig. 5B). Seasonal variation again includes an increase of chromatin material in spring and summer, and also an increase in lysosomal activity (Fig. 6A). In the lumen, myelinic structures and cellular debris are conspicuous (Fig. 6B).

The ductus epididymis is pseudostratified with nonciliated, columnar principal cells and scattered basal cells whose nuclei parallel the basal lamina (see Fig. 7). Some of the columnar principal cells are wider than others, but a distinct "narrow cell" as in mammals is not recognized, because all of the columnar cells seem similar in cytology. Microvilli are short and the elongate "stereocilia"

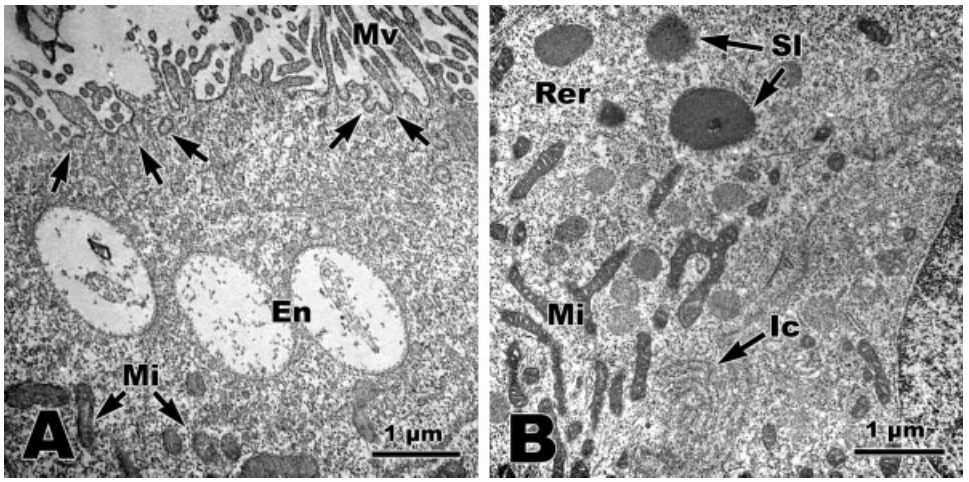

Fig. 5. TEM of the cytoplasm of the ductuli efferentes of *Seminatrix pygaea* collected in October. **A**: Apical pits (unlabeled arrows) in a non-ciliated cell and possible endocytic vacuoles. **B**: Perinuclear organelles. En, endocytic vacuoles; Ic, intercellular canaliculi; Mi, mitochondria; Mv, microvilli; Rer, rough endoplasmic reticulum; Sl, secondary lysosomes.

observed in mammals are absent. The cytoplasm of the principal cells of the October specimens is characterized by basal, oval nuclei with peripheral chromatin and nucleoli, numerous mitochondria (especially basally), narrow intercellular canaliculi, enlarged cisternae of endoplasmic reticulum, numerous electron-dense primary lysosomes (especially apically), conspicuous apocrine blebs, and an abundance of small vesicles (see Fig. 7).

The basal cells do not appear metabolically active, and contain only a few scattered mitochondria (Fig. 8A). The flocculent material seen in the ductuli epididymides does not appear as abundant, but the supranuclear Golgi complexes bud off vesicles which contain a product similar in density to the flocculent material (Fig. 8B). The vesicles empty their contents in the apical cytoplasm to form the apocrine blebs (Fig. 8C). Definite coated vesicles were not observed, but endocytosis is evidenced by various cellular inclusions including secondary lysosomes (Fig. 8D). Some sparsely granulated membranous structures associated with Rer contain vesicular inclusions (Fig. 8D).

The most noticeable seasonal variation occurs in a June specimen, in which the nuclei are heterochromatic and irregular (Fig. 9A). The abundant rough endoplasmic reticulum and Golgi complexes indicate that synthetic activity is still occurring (Fig. 9B). Myelin figures, which may be artifactual, are common in the lumen along the apical border, and the lumen is dense with a fine granular material (Fig. 9C,D).

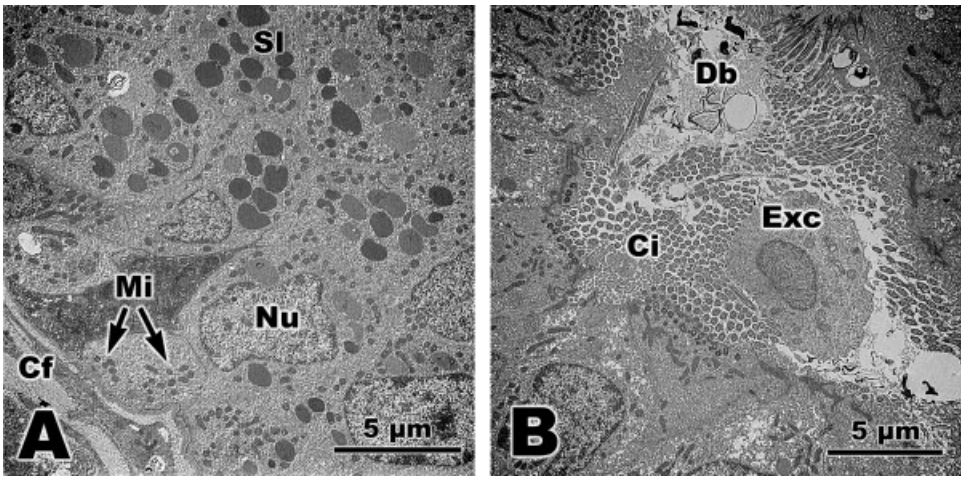

Fig. 6. TEM of the ductuli efferentes of *Seminatrix pygaea* collected in June. **A**: Cytoplasm of non-ciliated cells. **B**: Luminal border. Cf, collagen fibers; Ci, cilia; Db, debris; Exc, exocytosed cell; Mi, mitochondria; Nu, nuclei; Sl, secondary lysosomes.

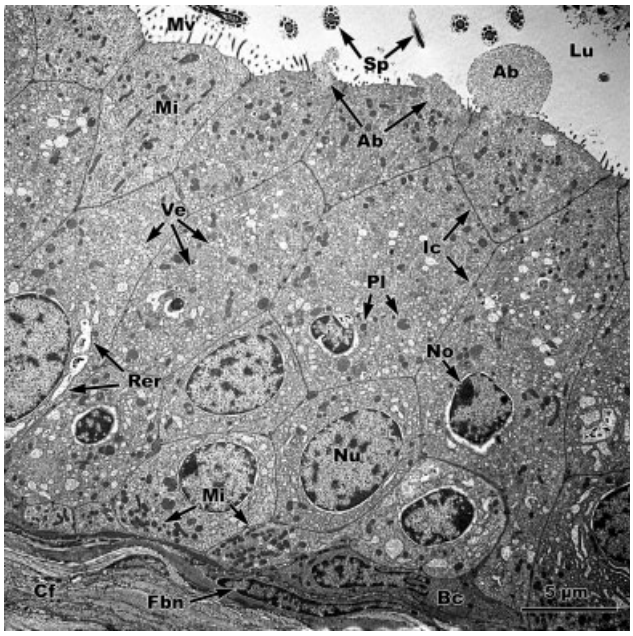

Fig. 7. TEM of the ductus epididymis of *Seminatrix pygaea* collected in October. Ab, apocrine bleb; Bc, basal cell; Cf, collagen fibers; Fbn, fibroblast nucleus; Ic, intercellular canaliculi; Lu, lumen; No, nucleolus; Nu, nucleus; Pl, primary lysosomes; Rer, rough endoplasmic reticulum; Sp, sperm; Ve, vesicles.

DISCUSSION

Seminatrix pygaea possesses a series of ducts passing from seminiferous tubules consisting of a simple layer of nonciliated cells that connect to tubules that contain both ciliated and nonciliated cells and pass sperm into the ductus epididymis. In other amniotes, the first ducts (whose epithelia may contain a single cilium in birds and mammals), are called the rete testis, and the tubules connecting the rete testis to the ductus epididymis are named the ductuli efferentes (Table 1; Jones, 1998). Volsøe (1944) in *Vipera berus* and *Natrix natrix*, Fox (1952) in *Thamnophis elegans* and *T. sirtalis*, and Saint Girons (1957) in *V. aspis* noted the division between proximal tubules with nonciliated cells ("ductuli efferentes" and "ductuli epididymides I") that connect to more distal ones with ciliated cells ("ductuli epididymides II"), but these tubules are similar to those of *S. pygaea*, and, in general, with other amniotes that have been studied (Table 1). Therefore, the traditional names used by Volsøe (1944), Fox (1952), and Saint Girons (1957) for snakes and other squamates should be changed to conform with those of other amniotes. As noted by Ilio and Hess (1994), the Anglicized form of ductuli efferentes is efferent ductules or ducts.

Variation does exist among amniotes in histology and ultrastructure of the anterior testicular ducts, and these differences may be important from functional and perhaps phylogenetic aspects (Table 1). For example, the ductuli efferentes of a lizard (Akbarsha et al., 2007), birds (Aire, 1980, 2002), and some mammals (Hess 2002) have been described as pseudostratified whereas the ducts are simple cuboidal to columnar in other groups. Most birds (Aire and Soley, 2003) and at least some mammals (Lesson, 1962; Dym, 1976) possess a single cilium on some cells in the rete testis, and birds also show this trait in the ductus epididymis (Aire, 2007). The ductuli efferentes of birds is differentiated into a proximal segment characterized by type I principal cells and a distal segment with type II principal cells; the type II cells are less involved in absorption and have fewer coated vesicles, vacuoles, and electron dense bodies (Aire, 1980, 2002). Crocodilians have a region termed the "ductuli epididymides" between the ductuli efferentes and the ductus epididymis (Guerrero et al., 2004); the ductuli epididymides possess secretory activity, which is lacking in the ductuli efferentes. Spermiophagy, perhaps as a response to pathological conditions, has been reported in mammals (Sinowatz et al., 1979; Hess 2002), birds (Aire, 2007), the lizard *Sitana ponticeriana* (Akbarsha et al., 2007), the turtle, *Chrysemys picta* (Holmes and Gist, 2004), and needs to be looked for in other taxa.

As in mammals and other amniotes that have been studied, the proximal efferent ducts of *Seminatrix pygaea* contain nonciliated principal cells that carry out absorptive and secretory functions without differentiation into two distinct cell populations (Hoffer et al., 1973; Ilio and Hess, 1994; Hermo et al., 1994). In mammals, testicular fluids undergo endocytosis into apical coated pits, which form tubules that merge into endosomes. Receptors in the endosomes are recycled to the cell surface whereas the endosomes transform to multivesicular bodies to secondary lysosomes (Hess 2002; Hermo and Robaire, 2002). Lipids are thought to be the eventual fate of digested material (Robaire and Hermo, 1988). The endocytoic organelles recognized in the nonciliated cells in the rete testis, ductuli efferentes, and ductus epididymis of *Seminatrix pygaea* correspond to those utilized in the absorptive processes described above for mammals.

Jones (1998) reported that the reptilian epididymis is composed of principal cells and basal cells, with the former being more columnar cranially and becoming cuboidal as the epididymis gradates into the ductus deferens. In *Seminatrix pygaea*, this transition is not so dramatic, as Sever (2004) still describes the principle cells of the ductus deferens as columnar.

I found no regionalized variation in the epididymis, but several investigators who have worked on lizards in the families Agamidae and Lacertidae have reported up to four regions corresponding to the initial segment, caput, corpus, and cauda of mammals (Desantis et al., 2002; Akbarsha et al.,

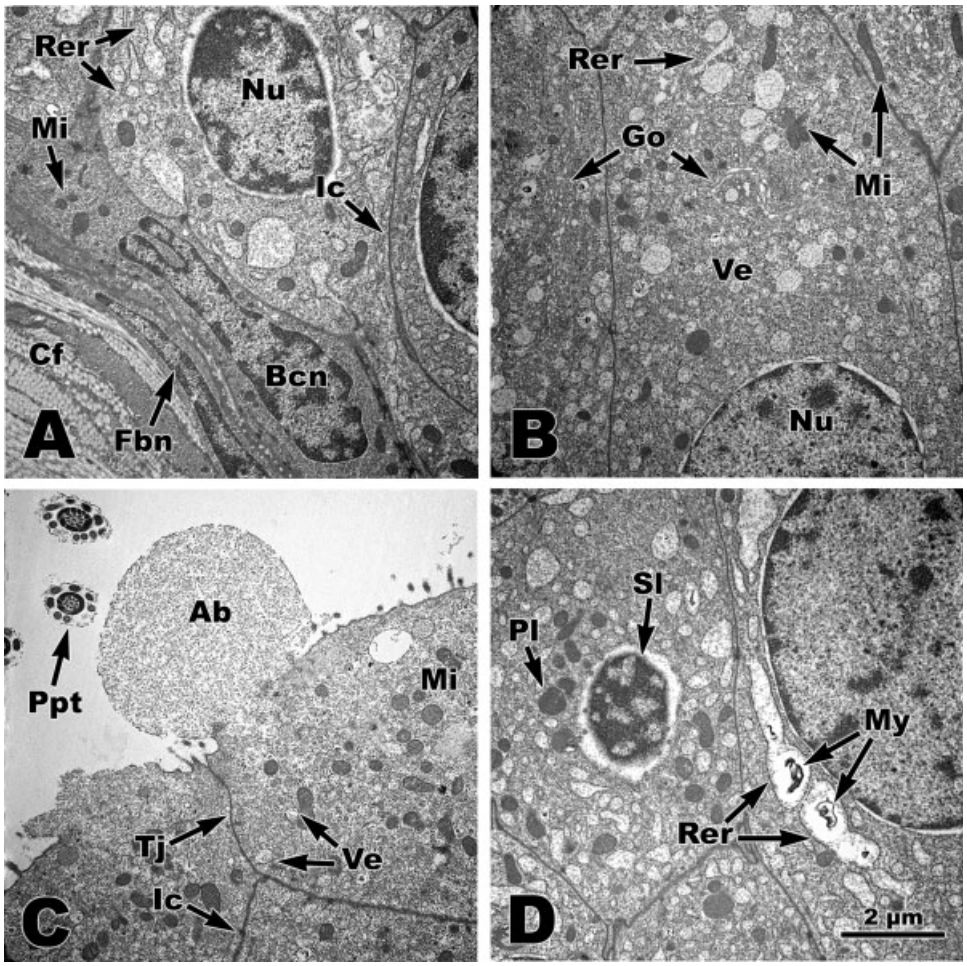

Fig. 8. TEM of the ductus epididymis of *Seminatrix pygaea* collected in October. Scale bar in (**D**) is for all images. **A**: Basal cell and adjacent principal cells. **B**: Supranuclear cytoplasm. **C**: Apical cytoplasm. D: Organelles associated with endocytosis. Ab, apocrine bleb; Bcn, basal cell nucleus; Cf, collagen fibers; Fbn, fibroblast nucleus; Go, Golgi complex; Ic, intercellular canaliculi; Mi, mitochondria; My, myelenic bodies; Nu, nucleus; Pl, primary lysosomes; Ppt, principle piece of the tail; Rer, rough endoplasmic reticulum; Sl, secondary lysosomes; Tj, tight junction; Ve, vesicles.

2006a,b, 2007). In addition, Akbarsha et al. (2006b) reported the same six kinds of cells (principal, basal, narrow, apical, clear, and intraepithelial leukocytes) in the epididymis of *Sitana ponticeriana* (Agamidae) as found in mammals, and a similar regional distribution for these cells. A focus for future research is to discover whether regionalization of cell types in the epididymis similar to that in mammals and reported in the lizard *Sitana ponticeriana* (Akbarsha et al., 2007) occurs widely in squamates.

DuFaure and Saint Girons (1984) examined histology of the epididymides of 89 species of squamates, representing 72 genera and 18 families, including 25 species of snakes. Much interspecific variation in secretory activity occurs, and perhaps this correlates with the variation observed in the few cytological studies. Lacertids and agamids possess clusters of dense apical secretory granules, whereas Dufaure and Saint Girons (1984) reported that secretory activity appears absent in snakes.

Seminatrix pygaea possesses a constitutive secretory pathway, similar to that of the proximal efferent ducts of mammals (Hoffer et al., 1973), in which the product is transported to the surface in small vesicles that Dufaure and Saint Girons (1984) would not have observed with light microscopy. Thus, the product is not concentrated or stored in granules while waiting for a neural or hormonal stimulus, but released as it is produced. In the case of *S. pygaea*, production seems to be year-around, although somewhat reduced in the summer. The product is both PAS+ and BB+, and therefore contains glycoprotein. Other studies demonstrate that secretions of the epididymis in lizards may contain lipid (Haider and Rai, 1987), proteins (Depeiges and Dufaure, 1980), and/or glycoproteins (Manimekalai and Akbarsha, 1992). Labate et al. (1997) found a variety of glycoconju-

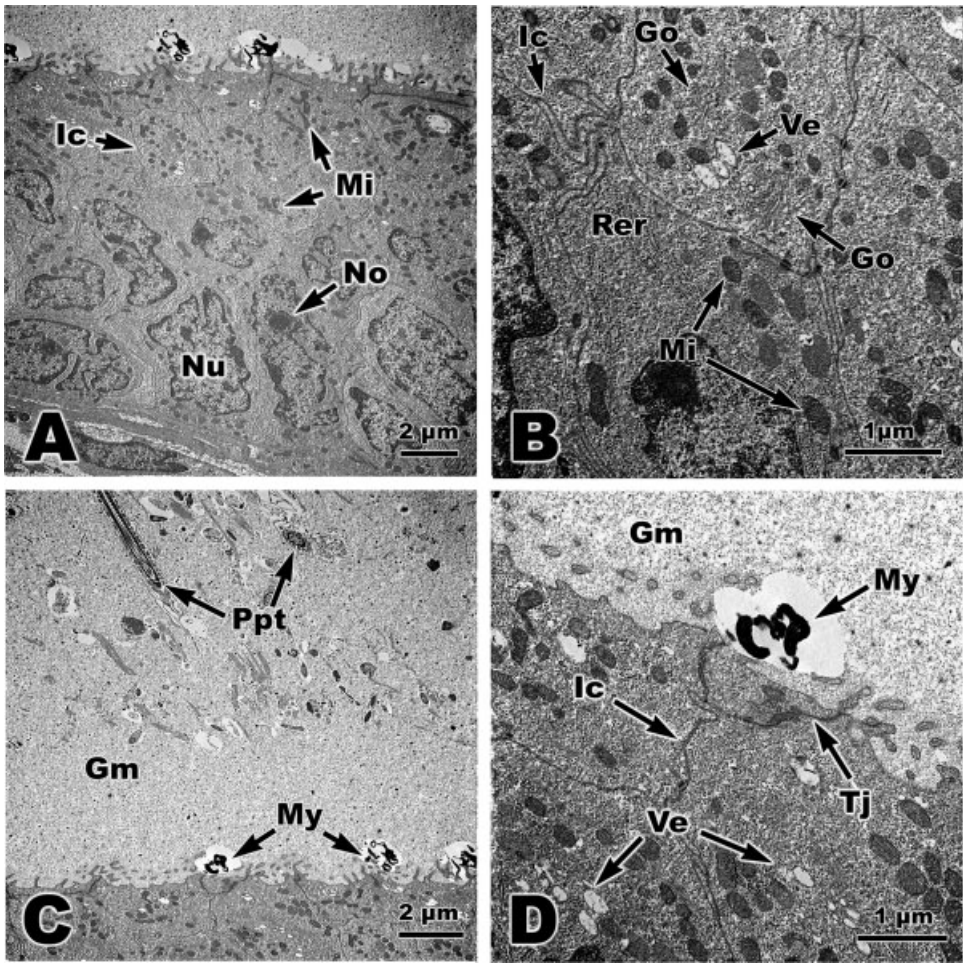

Fig. 9. TEM of the ductus epididymis of *Seminatrix pygaea* collected in June. **A**: Overview of epididymal cytoplasm and adjacent lumen. **B**: Perinuclear cytoplasm. **C**: Lumen. **D**: Luminal border. Gm, granular material; Go, Golgi complex; Ic, intercellular canaliculi; Mi, mitochondria; My, myelenic bodies; Ppt, principal piece of the tail; Rer, rough endoplasmic reticulum; Tj, tight junction; Ve, vesicles.

gates using lectin histochemistry in the ductuli efferentes of *Podacris sicula*, and the binding pattern varied seasonally. In the same species, Desantis et al. (2002) found that secretory cells along the length of the epididymis have 12 lectins binding to the secretions, with some regionalization. Although the function of the glycoconjugates in the epididymal secretory granules was not determined, perhaps they produce an environment for sperm storage and/or maturation, as known in mammals (Acott and Hoskins, 1981).

In mammals, much work exists on lectin histochemistry in the testicular ducts (e.g., Arenas et al., 1996, 1998; Parillo et al., 1997, 1998; Liu et al., 2000; Srivastav et al., 2004). Hermo and Robaire (2002) note that the principal cells of the mammalian epididymis are also involved in secretion of a large variety of proteins that are differentially secreted along its length. Golgi complexes and Rer are involved in primary lysosome production for the endocytosis function and vesicles for the secretory process.

The small vesicles release their products at the apical border in a merocrine fashion (Fig. 3D), but also may release their product within the cells to form the apocrine blebs. The granular material in the blebs resembles that of small vesicles adjacent to the blebs. Also, the content of the apocrine blebs is consistent with the appearance of the unorganized flocculent material found in supranuclear regions of some principal cells. The flocculent material may represent lipoid remnants of degraded material that passes into the blebs to be released into the lumen. Hermo and Robaire (2002) state that apical blebs in the mammalian epididymis consist of a variety of organelles, including dispersed Rer elements, polysomes, glycogen, and vesicles embedded in a homogeneous ground substance.

The ductuli efferentes are the only ducts of the male reproductive system of *Seminatrix pygaea* that possess ciliated cells. The cilia apparently help move sperm and luminal fluids toward

TABLE 1. General characteristics of the anterior testicular ducts of amniotes[a]

Region/Character	Snake	Lizard	Turtle	Crocodile	Bird	Mammal
Rete testis						
Epithelium	Simple squamous/ Cuboidal	Simple cuboidal	Simple squamous/cuboidal	Simple squamous/cuboidal	Simple squamous to columnar	Simple cuboidal/ columnar
Ciliated cells	No	No	No	No	Single cilium	Single cilium
Intratesticular	Yes	Yes	Yes	Yes	Yes	Yes
Extratesticular	Yes	Yes	Yes	Yes	Yes	Yes/no
Protein/CHO secretion	Yes	No report[b]	No report	No	Yes	Yes
Absorption	Yes	No report	No report	No report	Yes	Yes
Spermiophagy	No	No report	No report	No report	No	Yes
Large, indented nuclei	Yes	No report	No report	No report	Yes	Yes
Intercellular canaliculi	Labyrinthine	No report	No report	No report	Labyrinthine	Labyrinthine
Ductuli efferentes[c]						
Epithelium	Simple cuboidal	Pseudostratified	Simple columnar	Simple cuboidal/ columnar	Pseudostratified	Simple columnar/ pseudostratified
Highly convoluted	Yes	Yes	Yes	No report	No report	Yes
Ciliated cells	Yes	Yes	Yes	Yes	Yes	Yes
Cell types	2	3	3–4	3	3	up to 7
Protein/CHO secretion	Yes	No	Yes	Distally yes	Yes	Yes
Apocrine blebs	Yes	Yes	No report	No report	Yes	Yes
Absorption	Yes	Yes	Yes	Yes	Yes	Yes
Spermiophagy	No	Yes	No	No	Yes	Yes
Ductus epididymis						
Epithelium	Pseudostratified	Pseudostratified	Pseudostratified	Pseudostratified	Pseudostratified	Pseudostratified
Ciliated cells	No	No	No	No	Single cilium	No
Cell types	2	2-6	4	2	2	6
Zones	1	1-4	1	1	1	5
Protein/CHO Secretion	Yes	Yes	No	Yes	Yes	Yes
Apocrine blebs	Yes	Yes	No report	No report	Yes	Yes
Absorption	Yes	Yes	Yes	No	Yes	Yes
Spermiophagy	No	No	Yes	No	No report	Yes

[a]Data from Abd-Elmaksoud et al. (2009); Aire (1980, 2002, 2007), Aire and Soley (2003); Akbarsha et al. (2006a, 2006b, 2007); Desantis et al. (2002); Dym (1976), Goyal et al. (1992); Guerrero et al. (2004); Hermo et al. (1994); Hermo and Robaire (2002); Hess (2002); Holmes and Gist (2004); Jones (1998, 2002); Ilio and Hess (1994); Ladman et al. (1958); Lesson (1962); Mesure et al. (1991); Sinowatz et al. (1979).

[b]No report indicates the character has not been considered in publications on that taxon.

[c]In crocodilians, data include a region called the ductuli epididymides that connects the ductuli efferentes to the ductus epididymis.

the epididymis (Ilio and Hess, 1994). I neither observed any evidence of absorptive functions in ciliated cells as reported by Ilio and Hess (1994) in mammals nor any sign of secretory activity.

Future work should include studies of the ductus deferens. Jones (1998) proposes that reptiles and birds lack a ductus deferens, and the entire duct from the efferent ductules to the ureter should be called the ductus epididymis. Aire (2007) states that in birds the ductus deferens represents a different segment of the epididymal unit and thus supports Jones (1998). Sever (2004) limited his examination of the ductus deferens of *Seminatrix pygaea* to the caudal end, with special emphasis on the ampulla ductus deferentis. The ampulla is quite distinct, because of its highly folded epithelium. The main difference between the caudal ductus deferens and the ductus epididymides is the apparent lack of secretory activity in the former (Sever, 2004).

In summary, the histology and ultrastructure of the regions I call the rete testis and the ductuli efferentes in *Seminatrix pygaea* overall show a close similarity to ducts identified by those terms in other amniotes, and my working hypothesis is that the ducts are homologous in all amniotes. The old terminology established by Volsøe (1944) for ducts between the seminiferous tubules and epididymis should be abandoned for squamates, as they have for other sauropsids. As in other amniotes, the functions of these ducts include reabsorption of luminal fluid and secretion.

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