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Cover Page Footnote
I thank Dylan Ball for technical support. Lizard collection was authorized by a scientific collection permit from the Arkansas Game and Fish Commission.

Distal Urogenital Anatomy of Male Southern Coal Skinks, *Plestiodon anthracinus pluvialis* (Reptilia: Scincidae)

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Running Title: Distal Urogenital Anatomy of Male *Plestiodon anthracinus pluvialis*

Abstract

I investigated the morphology and histology of the distal urogenital anatomy of male southern coal skinks (*Plestiodon anthracinus pluvialis*) from reproductively active individuals collected in Arkansas in order to provide comparative information with recent studies on squamate urogenital anatomy. Specifically, I focused on the basic anatomy and positioning of posterior ducts in this skink, which included portions of the ductus deferens, the ampulla ductus deferentis, the sexual segment of the kidney, the ureter and collecting ducts, as well as aspects of the urodaeal chamber and urogenital papillae. I found a much reduced ampulla ductus deferentis comprising only 0.7 mm in length in the caudal region of the ductus deferens. The sexual segment of the kidney was well developed, being located in collecting ducts of the kidney proper, in walls of collecting ducts leading away from the kidney as well as within anterior portions of the ureter. The anterior dorsal recess of the urodaeum possessed epithelial crypts within a highly folded epithelium. Finally, a ductal triad (ductus deferens, ureter, and a single collecting duct) terminates at each orifice of the paired urogenital papillae. The distal urogenital anatomy of this scincid lizard revealed anatomical features similar to other species within the genus *Plestiodon*.

Introduction

Classical illustrations by Martin Saint Ange (1854), Brooks (1906), and Volsøe (1944) provided early macroscopic details of distal urogenital morphologies in male squamates (lizards and snakes). Although numerous studies have reported on male reproductive anatomy since these pioneer works (for comprehensive literature summaries, see Fox 1977; Trauth and Sever 2011; Rheubert *et al*. 2015), Gabe and Saint Girons (1965) provided the first detailed histological analysis of distal urogenital microanatomy. More recent investigations into the urogenital anatomy of male squamates, however, have launched a renewed interest in this region revealing several previously undescribed caudal micro-anatomical structures (Gribbins and Rheubert 2011; Trauth and Sever 2011; Rheubert *et al*. 2015). For example, Siegel *et al*. (2011) and Trauth and Sever (2011) recognized the uniqueness of a urogenital structure illustrated by Martin Saint Ange (1854)—the ampulla urogenital papilla (one of two complementary blind pouches)—which represents the terminal repository of products released from the distal urogenital tracts in North American colubrine snakes. Rheubert *et al.* (2015) also found these paired pouches in North American teiid lizards (family Teiidae) to the exclusion of all other lizards and families examined in their study. In addition, Trauth and Sever (2011) and Rheubert *et al*. (2015) were the first studies to document the broad range of variation in microstructure of the urogenital papillae in male squamates using scanning electron microscopy.

In an attempt to clarify nomenclature regarding the proximal testicular ducts (in snakes) for future studies, Sever (2010) synonymized all efferent ducts with those of higher amniotes. Included here were the rete testis, ductuli efferentes, ductus epididymis, and the ductus deferens (for these structures in a lizard, see Sever *et al*. 2013). Squamate distal urogenital ducts (i.e., ductus deferens, ampulla ductus deferentis, collecting ducts, ampulla urogenital papilla, and the ureter), on the other hand and with few exceptions, have received less histological attention compared to the proximal testicular ducts. Trauth and Sever (2011) and Rheubert *et al.* (2015) followed Sever's (2010) naming scheme in addressing urogenital structures in snakes and lizards, respectively.

The family Scincidae is a large, nearly cosmopolitan group of small-to-large lizards containing over 1,500 species (Vitt and Caldwell 2014). Skinks are widespread in Arkansas (Walley 1998; Trauth *et al*. 2004; Powell *et al*. 2016), and there are six species in two genera (*Plestiodon* [= *Eumeces*] and *Scincella*). Trauth *et al*. (1987) focused on female urogenital anatomy of

Plestiodon laticeps and only briefly described male cloacal anatomy. Trauth (1994) reported on the reproductive cycles of two skink species (*Plestiodon anthracinus pluvialis* and *Plestiodon fasciatus*) from Arkansas and provided light photomicrographs of the testicular and epididymal histology. Rheubert *et al.* (2015) described aspects of the male urogenital anatomy in *Plestiodon fasciatus* and *Scincella lateralis* and included brief comments and scanning electron micrographs of the urogenital papillae in *P. a. pluvialis* and *P. laticeps.*

In the present study, I describe the distal urogenital anatomy of male southern coal skinks (*Plestiodon anthracinus pluvialis*) using macrophotography and histology. I also compare these findings with those published on other scincid lizards reported in Rheubert *et al.* (2015).

Materials and Methods

For light microscopy (LM), I dissected distal urogenital tissues from two male southern coal skinks previously deposited in the Arkansas State University Museum of Herpetology (ASUMZ 8950 and 24012). The skinks had been collected in Arkansas by my students or me. I also utilized three additional male specimens (ASUMZ 12727, 16392, and 30659) for macroscopic analysis and macrophotography of urogenital structures. All five specimens had been euthanized with an intra-pleuroperitoneal injection of sodium pentobarbital, and internal organs were then fixed using abdominal injections of 10% formalin. Other skinks utilized (*Scincella lateralis*, ASUMZ 20335; *Plestiodon laticeps*, ASUMZ 31171) are museum specimens preserved in 70% ethanol.

I necropsied distal urogenital tissue segments (approximately, 10 mm in length) and placed these into vials of 70% ethanol. Then, I followed standard histological techniques to prepare tissues for LM following the paraffin embedding procedures outlined in Presnell and Schreibman (1997). In brief, these steps included dehydration using a graded series of ethanol solutions (70 to 100%), clearing to 100% xylene, infiltration overnight in a paraffin oven (56°C), embedding with paraffin into 23 mm square plastic molds (tissue positioned upright in a cranial-to-caudal axis), sectioning with a rotary microtome into 10 µm serial strips (affixed onto glass microscope slides coated with Haupt's adhesive prior to floating strips in 2% formalin on a slide warmer), and staining using Pollak trichrome stain for the enhancement of connective tissues and muscle. Cover slips were adhered to microscope slides with Permount© (Fisher Scientific Products).

For slide photomicroscopy, I used a Leica MC 120 HD camera atop a Leica DM 2000 LED compound light microscope. For macrophotography, I used either a Canon T4i digital single lens reflex camera fitted with a macro lens or the aforementioned camera atop a Leica M 80 stereomicroscope.

All descriptions of urogenital structures follow the terminology found in either Trauth and Sever (2011) or Rheubert *et al.* (2015). Microscope slides are currently in my possession.

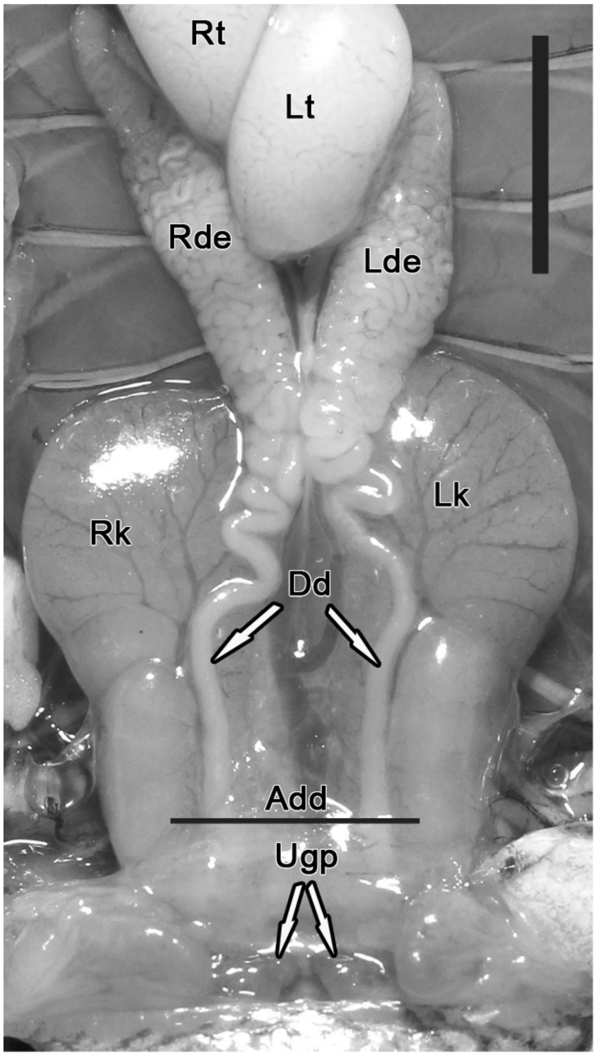

Figure 1. Urogenital system of a recently sacrificed scincid lizard (*Plestiodon fasciatus*, ASUMZ 32717) as adapted with modifications from Fig. 9.1D in Rheubert *et al*. (2015). Scale bar at upper right = 5 mm; Add, ampulla ductus deferentis (line below Add approximates the location of this structure within the ductus deferens); Dd, ductus deferens; Lde, left ductus epididymis; Lt, left testis; Rde, right ductus epididymis; Rt, right testis; Rk, right kidney; Lk, left kidney; Ugp, urogenital papilla.

Results

Gross Morphology

A representative scincid urogenital anatomy (ventral view) of a reproductively active male lizard (*Plestiodon fasciatus*) is shown in Fig. 1 and a brief description is provided here. The paired testes appear cream-to-white in color with the right testis lying more cranial compared to the left. Each testis is flanked laterally by an adrenal gland (best seen in figure as a thin line of tissue between the left testis and left ductus epididymis) and the convoluted efferent tubules of the ductuli efferentes (not identified in Fig. 1), which eventually converge into a common, highly looped, ductus epididymis. The ductus epididymis becomes less looped and straightens along the anterior ventromedial surface of the kidney as it becomes the anterior ductus deferens. Anterior urogenital structures of *Plestiodon anthracinus pluvialis* (starting with the right ductus epididymis) are shown in Fig. 2. The posterior ductus deferens continues as a straight duct on both sides (Figs. 1, 2). Not pictured in Figs. 1 and 2 is the ureter, which lies dorsomedial to the posterior ductus deferens.

Light Microscopy

The distal urogenital anatomy of *Plestiodon anthracinus pluvialis* is shown in Figs. 3 – 5 and is illustrated histologically from approximately 2 mm of tissue (see black box region in Fig. 2). For most of its length, the ductus deferens (Dd) is a circular-to-oblong linear duct and is uniformly lined with either a short or tall pseudostratified columnar epithelium (Fig. 5A); however, as the Dd leads caudally and nears the cloacal region, a short enlargement (approximately 0.7 mm in length) of the duct, the ampulla ductus deferentis (Add), becomes apparent (Figs. 3A; 4A, B; 5B). This expansion is characterized by a slight increase in the diameter of the duct and by a circumferential folding of its epithelial lining. These diagnostic histological and morphological features of the Add soon disappear, and the Dd regains its caudal structural appearance as observed in the more anterior regions of the duct (e.g., Fig. 5A).

Initially, the ureter (Ur) varies little with respect to its anatomical position lying ventral to the kidney and adhering closely with the dorsomedial surface of the Dd (Figs. 3A, B; 4A, B). In addition, its pseudostratified epithelium may appear folded (Fig. 5D) throughout much of its length. Caudally, however, the Ur moves to reside laterally (Fig. 3C) and then ventromedially to the Dd (Fig. 3D; 4D, E). As the Ur reaches the urodaeal

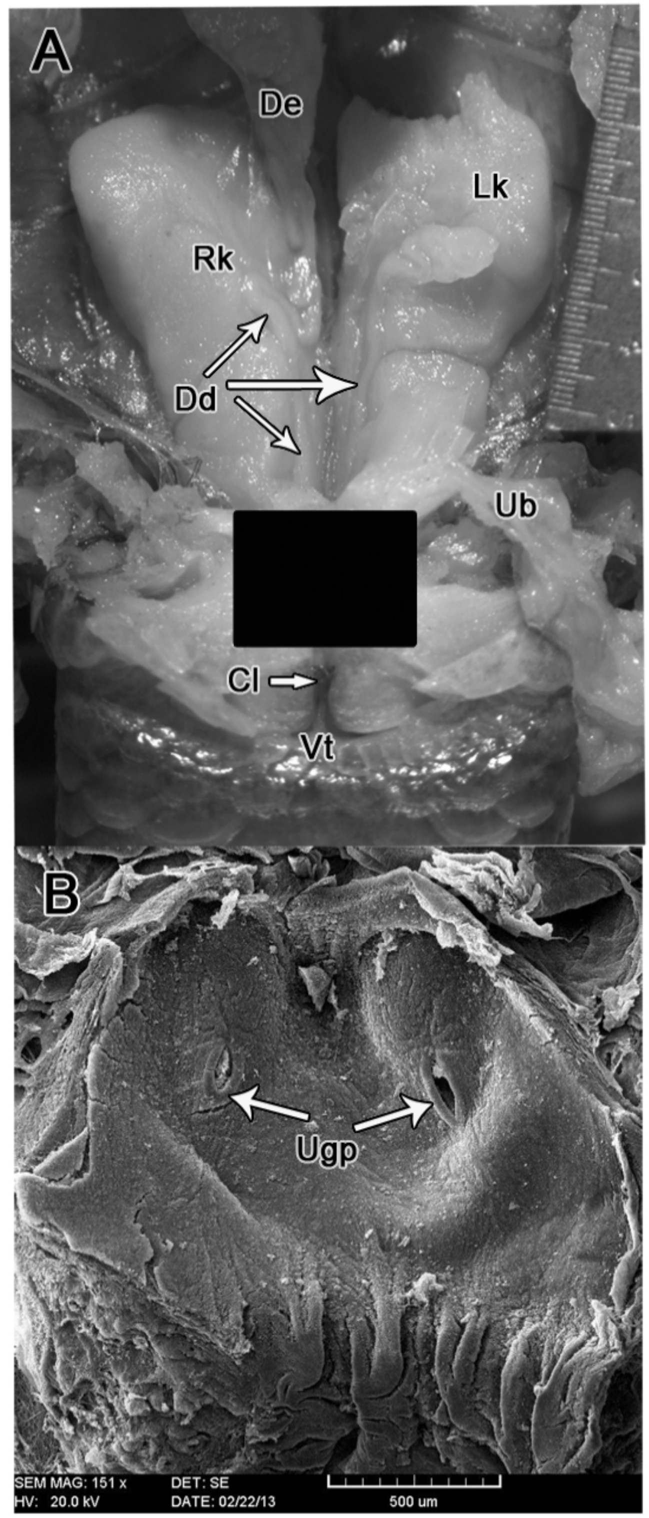

Figure 2. Macroanatomy (A) and scanning electron micrograph (B) of distal urogenital structures in *Plestiodon anthracinus pluvialis* (A, ASUMZ 12727; B, ASUMZ 16392; image adapted from Fig. 9.26F in Rheubert *et al*. 2015). Abbreviations same as in Fig. 1. Scale bar in upper right corner of A in mm; Ub, urinary bladder; Ugp, urogenital papilla; Cl, cloaca. Black box of A incorporates the portion of the distal urogenital anatomy illustrated in Figs. 3, 4.

region of the cloaca (Fig. 4F), it briefly receives sperm from the Dd and immediately redirects all urogenital products found within the distal urogenital ducts into the cloaca through the orifice of a urogenital papilla (Figs. 2B; 3F; 4F).

The sexual segment of the kidney (Ssk) is clearly evident at all levels of the kidney (Figs. 3–5). The Ssk is variable in structure, but is distributed throughout each kidney primarily as part of the collecting ducts of nephrons. Several collecting ducts (Cd) lead away from the kidney and can be seen residing dorsal to the Dd and Ur (e.g., Fig. 3A – C; 4B). They initially appear oblong or flattened (Figs. 3A, B; 4B) but will eventually enlarge and merge into a single, spherical duct lying dorsal to the Dd (Fig. 3C). This duct-like channel can be seen adjacent to the Dd along its dorsal and lateral surfaces (Fig. 3D, E). All three ducts (Dd, Cd, and Ur) are encased by a common sheath of connective tissue (Figs. 3C–E; 4E; 5E, F) and can be termed the urogenital ductal triad.

At approximately the level of the initial clustering of Cd, Dd, and Ur, two additional cavities appear (Figs. 3C; 4E). These cavities are the anterior dorsal recesses (Adr), which are anterior extensions of the urodaeum. These cavities are initially paired (Fig. 3C), but will eventually unite medially (Fig. 4E) and merge with the intestine.

Discussion

Ductus deferens

Rheubert *et al.* (2015) examined the distal urogenital anatomy in 7 species of lizards; 2 of these were skinks (*Plestiodon fasciatus* and *Scincella lateralis*). The pseudostratified epithelium of the Dd of *Plestiodon anthracinus pluvialis* was similar to that found in both species. Unlike *Scincella lateralis*, however, the Ur and Dd in *P. a. pluvialis* and *P. fasciatus* unite near the orifice of the urogenital papilla (see ***Urogenital papillae***).

Ampulla ductus deferentis

Only recently have researchers identified an Add within the Dd of squamates. Sever (2004) and Akbarsha *et al.* (2005) were the first studies to describe the Add in snakes and lizards, respectively. Trauth and Sever (2011) provided diagnostic morphological features of the micro-anatomies of the Add in seven colubrine snakes and noted the variable complexity of the epithelial folding of the ampulla. One species of watersnake (*Nerodia cyclopion*) exhibited a diverse branching pattern of the ductal epithelium. Rheubert *et al.* (2015) reported on the presence of an Add in additional lizards, including *Scincella lateralis* and four phrynosomatids (*Cophosaurus texanus*, *Sceloporus consobrinus*, *Holbrookia propinqua*, and *Phrynosoma cornutum*). The Add of *Plestiodon anthracinus pluvialis* is similar to that exhibited by these four phrynosomatids (presence of varying degrees of folding micro-anatomies of the epithelium). Thus far, however, the most complex Add epithelial morphology for any lizard was found by Rheubert *et al.* (2015) in *S. lateralis* (Fig. 4C).

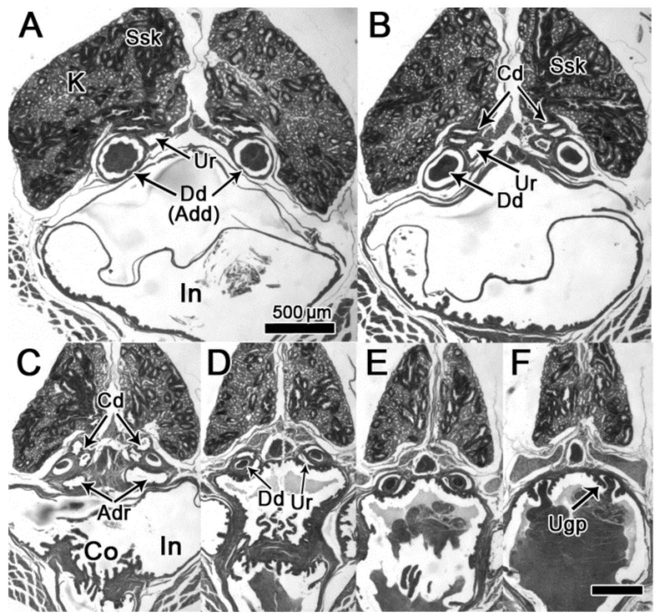

Figure 3. Light micrographs of the distal urogenital anatomy in *Plestiodon anthracinus pluvialis* (ASUMZ 8950) as revealed by a cranial-to-caudal series of histological sections (A–F). A. Section through the urogenital complex at the level of the ampulla ductus deferentis (Add). B. Section posterior to A showing sexual segment of the kidney (Ssk), collecting ducts (Cd), the ureter (Ur), and the ductus deferens (Dd). C. Section showing all structures in B, but also including the anterior dorsal recess of the urodaeum (Adr), the intestine (In), and the beginning of the coprodaeum (Co). D. Section just anterior to the cloacal chamber showing the relative size and position of the Cd, Dd, and Ur (see also Fig. 4E). E. Section just anterior to the urogenital papillae (Ugp). F. Section through the orifices of the paired Ugp showing release of sperm and other urogenital products; scale bar = 500 µm. Co, coprodaeum. Other abbreviations as in previous figures.

Sexual segment of the kidney

The morphology of the Ssk in lizards has been well illustrated (Prasad and Reddy 1972) and well documented in many snake and lizard families (Rheubert *et al*. 2015). Rheubert *et al.* (2015) also provided a table and a phylogenetic character map summarizing the morphological region of the Ssk within the nephron in squamates. In Scincidae, the Ssk is distributed within the Cd and in the Ur. The Ssk is characterized by tall columnar epithelial cells with basal nuclei (Fig. 5A, C). Large secretory granules are released as a merocrine secretion into the lumina of these ducts. In *Plestiodon anthracinus pluvialis*, the Ssk is similar to other skinks and to squamates, in general.

Collecting ducts

The Cd are conspicuous ducts that reside near or adjacent to the Ur and Dd. Together, these three structures become a ductal triad as they reach their termination point at the orifice of the urogenital papilla in all skinks studied thus far. The Cd in *Plestiodon fasciatus*, *Plestiodon anthracinus pluvialis* (Fig. 5A), and *Scincella lateralis* may exhibit epithelial folding and cytoplasmic features characteristic of the Ssk. In *P. a. pluvialis*, however, the folding diminishes as the duct nears the cloaca (Fig. 5E, F).

Anterior dorsal recess of the urodaeum

Within squamates, the Adr was first described in snakes as anterior projecting cavities of the urodaeum (Trauth and Sever, 2011); these cavities were then described in lizards (Rheubert *et al.* 2015). In *Plestiodon fasciatus*, the epithelium of the Adr is highly convoluted and contains numerous primary and secondary crypts. An electron micrososcopic analysis of these crypts revealed an orderly arrangement of sperm clustered within these spaces (Rheubert *et al.*, 2015). Although *Plestiodon anthracinus pluvialis* also possesses crypts (Fig. 5F), no sperm were detected in them during the present study. No Adr was observed in *Scincella lateralis*.

Ureter

The Ur lies medially to the Dd throughout most of its length in *Plestiodon anthracinus pluvialis*. Also, a highly folded epithelium was found within the anteriormost regions of the duct (Fig. 5D); this was attributed to Ssk development. In more caudal regions, the Ur loses most of its Ssk features (Fig. 5E, F), becomes flattened, and moves to reside in its terminal position ventromedial to the Dd (Fig. 4E). In contrast, *Plestiodon fasciatus* exhibited a relatively large Ur (called a secondary Cd by Rheubert *et al.* 2015), and it retained Ssk morphological features. Unlike both of these skinks, the Ur in *Scincella lateralis* opened separately into the cloaca.

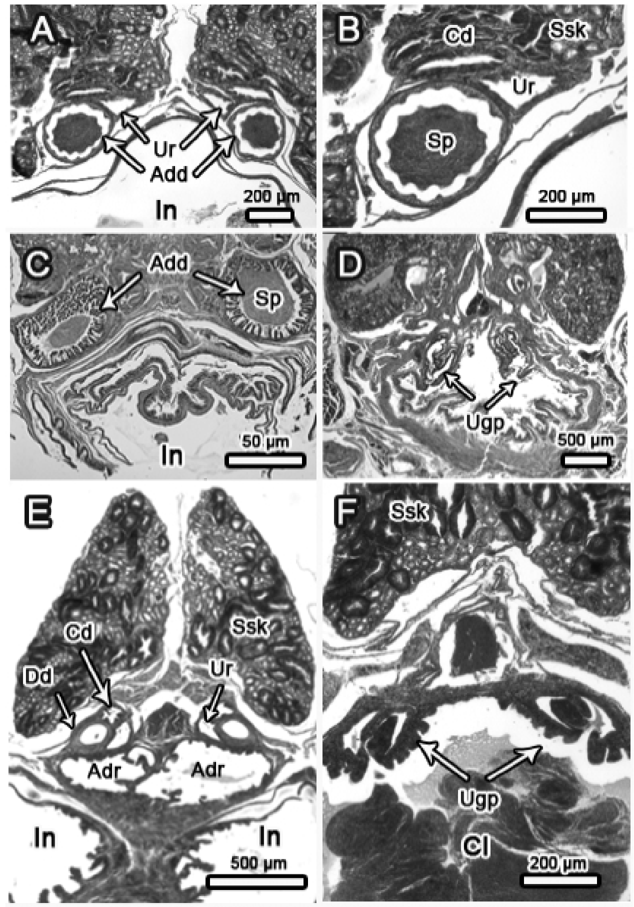

Figure 4. Light micrographs of urogenital structures in *Plestiodon anthracinus pluvialis* (A, B, E, and F; ASUMZ 8950), *Scincella lateralis* (C, ASUMZ 20335), and *Plestiodon laticeps* (D, ASUMZ 31171). Sp, spermatozoa; Cl, cloaca. Other abbreviations as in previous figures.

Urogenital papillae

The termination point for release of all ductal components in male squamates is the urogenital papilla (Ugp), which may be either a single medial structure or paired bilateral structures along the dorsal wall of the urodaeum of the cloaca (Trauth and Sever 2011; Rheubert *et al*. 2015). A detailed description and illustration of a generalized Ugp morphology (and its surrounding tissues) is found in Trauth and Sever (2011). In addition, the highly variable external micro-anatomy of the Ugp is best viewed using scanning electron microscopy as depicted in Trauth and Sever (2011) for snakes and Rheubert *et al*. (2015) for lizards. In all skinks studied thus far, there are two Ugp. In *Plestiodon anthracinus pluvialis* the Ugp are much reduced in size and complexity, and they are little more than small ridges or lips surrounding an orifice (Figs. 2B; 3D – F; 5E, F). In comparison, other skinks, like

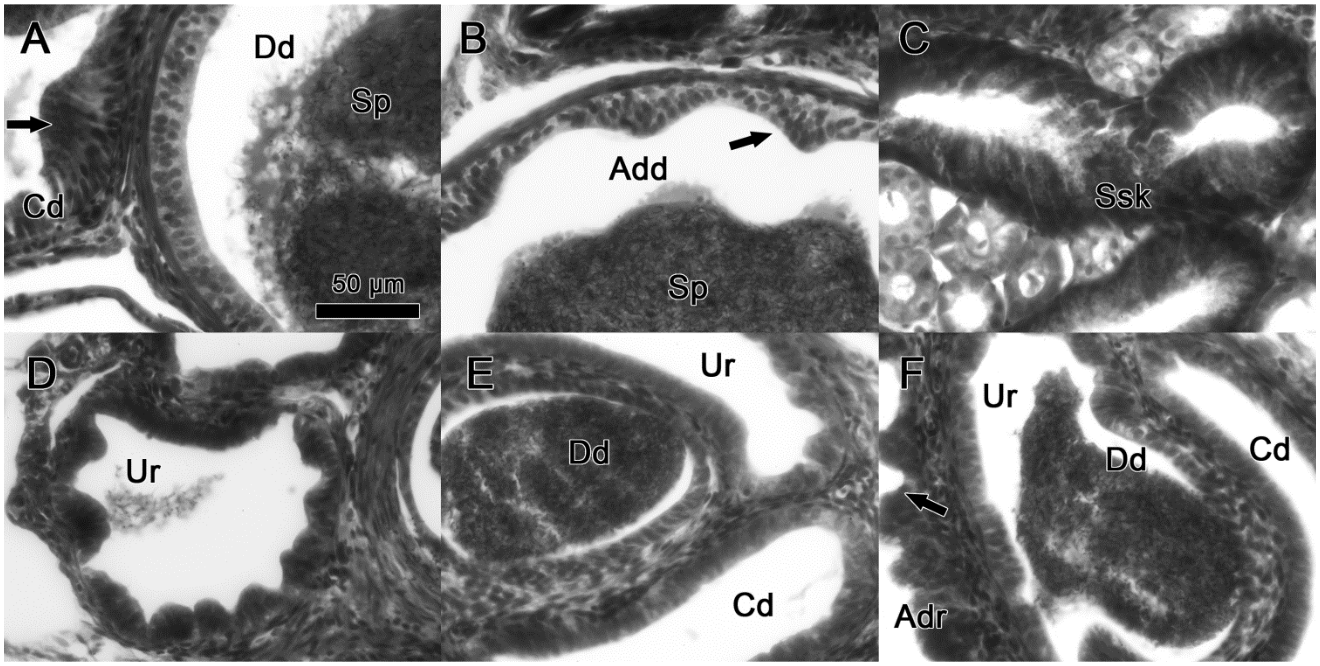

Figure 5. Light micrographs of epithelia of urogenital structures in *Plestiodon anthracinus pluvialis* (ASUMZ 8950). A. Section through the ductus deferens (Dd) and an adjacent collecting duct (Cd) showing epithelial pseudostratification; the Cd possesses tall irregular cells containing secretory granules (arrow) characteristic of the sexual segment of the kidney (Ssk). B. Section through the Dd posterior to A in the region of the ampulla ductus deferentis (Add). C. Section of kidney tubules showing a region of the Ssk. D. Section through a variable epithelium of the ureter (Ur). E. Section encompassing the Dd, Cd, and Ur nearing the urodaeal region (see Fig. 4E) revealing low heights of the epithelia. F. Section posterior to E through the Dd, Cd, and Ur. Scale bar in A the same for B–F; Sp, spermatozoa.

Plestiodon fasciatus (Fig. 1), *P. laticeps* (Fig. 5D), and *Scincella lateralis* (Rheubert *et al*. 2015), have Ugp that are more bulbous (as seen in reproductively active individuals), and they occupy more physical space within the cloaca.

The internal anatomy of the Ugp in *Plestiodon anthracinus pluvialis* reveals a ductal triad that varies little from the morphology found in *Plestiodon fasciatus.* In these two skinks, the Dd dumps sperm directly into the Ur (Fig. 5F), which then opens immediately into the Cd (Fig. 4F). All ductal materials are then released into the cloaca through a single common chamber (Fig 3F).

Acknowledgments

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