



## Morphological and histological characterization of sexual segment of the kidney in *Notomabuya frenata* (Cope, 1862) and *Aspronema dorsivittatum* (Cope, 1862) (Squamata, Mabuyidae)

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### ABSTRACT

The kidneys in two viviparous species of Neotropical lizards, *Notomabuya frenata* and *Aspronema dorsivittatum* (Mabuyidae), were investigated by light and scanning electron microscopy in order to determine the presence of the sexual segment of the kidney (SSK) and to study its morphology. The individuals used in this study belong to the Herpetological Collection of the Herpetology Laboratory - Reptiles of the Federal University of Juiz de Fora (CHUFJF-Reptiles) and they were collected between the years 2008 and 2012 from the Cerrado region in the state of Minas Gerais, Brazil. The SSK was present only in sexually mature males (with sperm in the testes / epididymis), whereas it was absent in sexually immature males. The nephron in both species consists of renal corpuscle, proximal convoluted tubule, distal convoluted tubule, collecting duct and sexual segment of the kidney. The SSK of the analyzed species were coated with a simple columnar epithelium, with high cells, basal nucleus and in the apical portion innumerable secretory granules. This study adds to the knowledge on reproductive biology and structures related to reproductive strategies of both lizard species and viviparous Neotropical lizards.

**Key words:** Accessory organ, reproduction, secretion granules, skins, urogenital system.

### INTRODUCTION

The sexual segment of the kidney (SSK) was first observed by Gampert (1866) when studying the

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urinary tube of *Natrix natrix* (Linnaeus, 1758) (= *Tropidonotus natrix*) (Colubridae). Subsequently, in the same species, Heidenhain (1868) showed the granular nature of the secretion that filled the cytoplasm of the cells that constituted this differentiated portion of the urinary tube. In analyzing the kidneys of *Natrix maura* (Linnaeus,

1758) (= *Tropidonotus viperinus*) (Colubridae) and *Vipera aspis* (Linnaeus, 1758) (Viperidae), Tribondeau (1902) also noted the presence of an enlarged portion in the urinary tube. The diameter varied among the species studied. However, Regaud and Policard (1903a, b) were the first researchers to correctly interpret this particular feature of the kidneys of Squamata, through finding that this enlarged portion of the urinary tube existed only in the males of some species of snakes. Because these authors suspected that this segment of the kidneys might have a reproductive function, they named it the “sexual segment”. In addition to assigning a sexual meaning to the distal portion of the renal tubules, these authors reported that seasonal oscillation could occur in this structure, which they correlated with cyclical variations in the reproductive organs.

The SSK is a feature observed in the kidneys of Squamata and is characterized by enlargement of a portion of the nephron and/or ureter (Regaud and Policard 1903a, b, Sanyal and Prasad 1966, Gabri 1983, Mendes et al. 2009, Rheubert et al. 2014). It is usually not present in females and immature males of Squamata (Kühnel and Krisch 1974, Del Conte and Tamayo 1973, Sever and Hopkins 2005, Rheubert et al. 2014).

The function of the SSK remains unclear (Misra et al. 1965, Reddy and Prasad 1970, Prasad and Reddy 1972). However, it is believed that the SSK is homologous to the seminal vesicles of mammals, since both respond similarly to androgen hormones (Mendes et al. 2009). The SSK is indispensable for copulation and activities such as spermatogenesis and testosterone production, since it has functions associated with production of pheromones and secretions from the ampullae and ductus deferens, separation of urine from semen inside the cloaca, sperm nutrition and semen retention in the female’s cloaca, along with providing a buffer at the female genital opening and thus preventing copulation with other males (Sever et al. 2002, Sever and Hopkins

2005, Rheubert et al. 2014). In this way, studying the SSK helps in understanding the reproductive biology and reproductive strategies of reptiles.

Studies on the reproductive biology of species popularly known as “skinks” have revealed peculiar characteristics of their reproduction and life history. They have features similar to the eutherian mammalian placenta, such as a structure with placentrophic viviparity, early breeding, and pregnancy ranging in duration from 8 to 12 months (Vitt and Blackburn 1991, Blackburn and Vitt 1992, Silva and Araújo 2008, Hernandez-Franyutti and Uribe 2012).

Although the biological and ecological aspects of the skinks have been studied for decades in Brazil, the number of studies elucidating the biology and reproductive strategies of these lizards remains small in relation to the level of biodiversity present in this country. Some reproductive aspects of *Notomabuya frenata* (= *Mabuya frenata*) (Cope, 1862) and *Aspronema dorsivittatum* (= *Mabuya dorsivittata*) (Cope, 1862) have previously been studied (Vitt and Blackburn 1991, Rocha and Vrcibradic 1999), such as the reproductive cycle, litter size, cycle of fatty bodies and minimum size for sexual maturity (Vrcibradic and Rocha 1998, Vrcibradic et al. 2004). However, there are no studies relating to morphological and histological characterization of the urogenital system of these two species.

Given the importance of the sexual segment of the kidney for reproductive and physiological activities of Squamata and the scarcity of studies addressing this structure in Neotropical viviparous species, the present study aimed to characterize the SSK of *Notomabuya frenata* and *Aspronema dorsivittatum*, morphologically and histologically.

#### MATERIALS AND METHODS

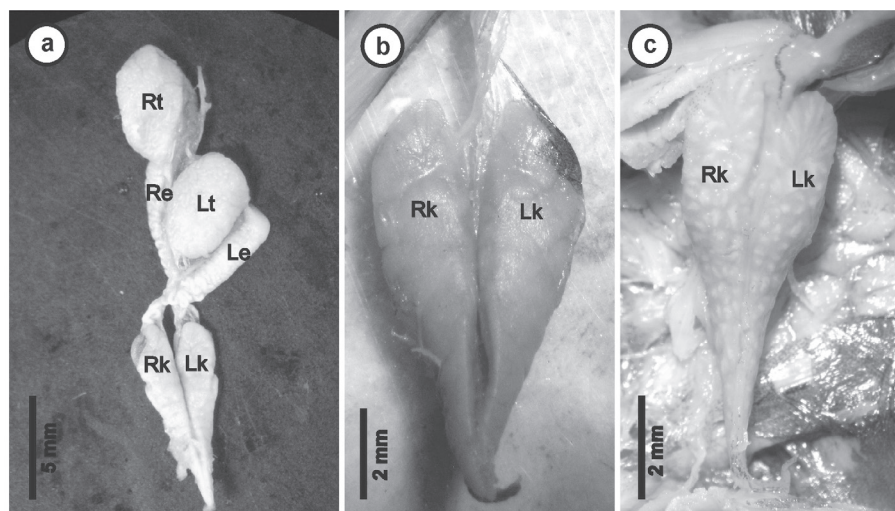
This study included eight individuals of *N. frenata* (five adult males and two young males) and eight

individuals of *A. dorsivittatum* (five adult males and two young males), belonging to the reptile section of the Herpetological Collection of the Federal University of Juiz de Fora (CHUFJF-Reptiles). The lizards were collected during previous studies in the Cerrado biome (Novelli et al. 2012, Lucas et al. 2016), in the Reserva Biológica do Boqueirão (21°20'47" S; 44°59'27" W), municipality of Ingaí, Minas Gerais, Brazil, between September 2008 and January 2012. According to the records at CHUFJF-Reptiles, all of these individuals were fixed in 10% formalin and stored in 70% ethanol.

During necropsies on these individuals, part of the urogenital system (kidneys and testes) of each individual was placed in 10% formalin, to be subjected to histological techniques. Before processing, the tissue sample (Figure 1) was cleaved in cross-section or kept whole for processing. The kidney of two individuals of *N. frenata* (right portion) and two individuals of *A. dorsivittatum* (left portion) were removed for processing according to the technique used for analysis in scanning electron microscopy. For the optical microscopy analysis, the tissue from the urogenital system was dehydrated in increasing concentrations of alcohol

(80%, 90% and 100%), cleared in xylene and embedded in paraffin. Subsequently, histological microtome blocks were cut into serial sections with a thickness of 4  $\mu\text{m}$  (Tolosa et al. 2003). The slides were stained with hematoxylin-eosin (H&E), periodic acid-Schiff (PAS), Alcian blue (AB) at pH 2.5% (Rheubert et al. 2011) and Gomori's trichrome. All histological processing was carried out in the Histology Laboratory, Department of Morphology, Institute of Biological Sciences, Federal University of Juiz de Fora (UFJF), Juiz de Fora, Minas Gerais. Analyses on the material and photo documentation were performed using an Olympus BX41 microscope with digital photographic equipment (Canon A3100S) and an ocular micrometer coupled to this microscope.

For the scanning electron microscopy analyses, one kidney from each sexually mature male (two individuals of *N. frenata* and two individuals of *A. dorsivittatum*) was fixed in 10% formalin and dehydrated in increasing concentrations of ethanol (70%, 80%, 90%, 96% and 100%). After dehydration, the material was subjected to 97% 1,1,1,3,3,3-hexamethyldisilazane until dry. The material was mounted on stubs using adhesive tape,



**Figure 1** - Urogenital system of *Aspronema dorsivittatum* (a). Kidney of *Notomabuya frenata* (b) and of *A. dorsivittatum* (c). Right testicle (Rt); Left testicle (Lt); Right epididymis (Re); Left epididymis (Le); Right kidney (Rk); Left kidney (Lk).



metallized with gold, and subsequently analyzed using an FEI Quanta FEG microscope operating at 10 kV.

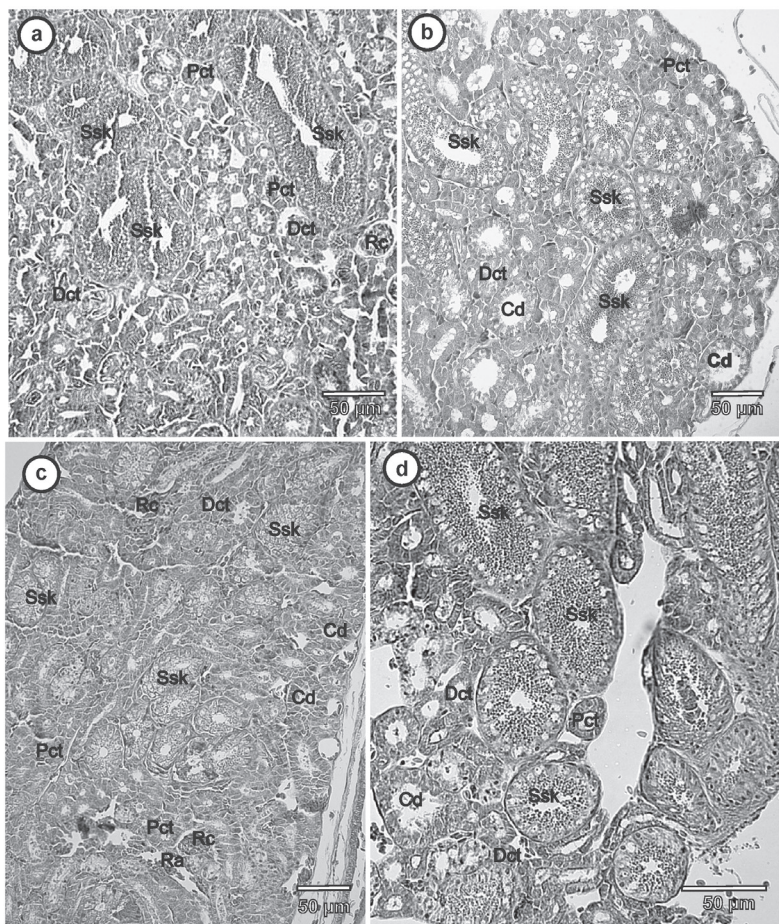
## RESULTS

The kidneys of both species were located in the posterior portion of the trunk, in the studied individuals. Regarding external morphology, the kidneys of *N. frenata* presented a smooth texture whereas in the kidneys of *A. dorsivittatum*, a granular texture was observed (Figure 1b, c).

Through the microscopic analysis of the kidneys of *N. frenata* and *A. dorsivittatum* it was possible to infer that the kidneys organize

themselves serially; that is, there is no separation between cortex and medulla (Figure 2).

The renal parenchyma (Figure 2a, c) was characterized by well-defined nephron components (renal corpuscles, proximal and distal convoluted tubules and collecting duct). The renal corpuscles consisted of a glomerulus and corpuscular space (Figure 2). The proximal convoluted tubules (PCTs) were composed of a simple cuboidal epithelium with strong acidophilic cells that were stained more intensely at the edges (or fluted edges or brush border), which was consistent with the presence of microvilli (Figure 2). The distal convoluted tubules (DCTs) were distinguished



**Figure 2** - Light microscopy kidney sections in *Notomabuya frenata* (a, b) and *Aspronema dorsivittatum* (c, d) stained with hematoxylin and eosin (H&E). Renal corpuscle (Rc); Proximal convoluted tubule (Pct); Distal convoluted tubule (Dct); Collecting duct (Cd); Sexual segment of the kidney (Ssk).

from the PCTs due to their smaller diameters and simple cuboidal epithelial lining, and were poorly acidophilic (Figure 2). The collecting ducts (CDs) were lined with low columnar epithelia, which indicated secretory activity.

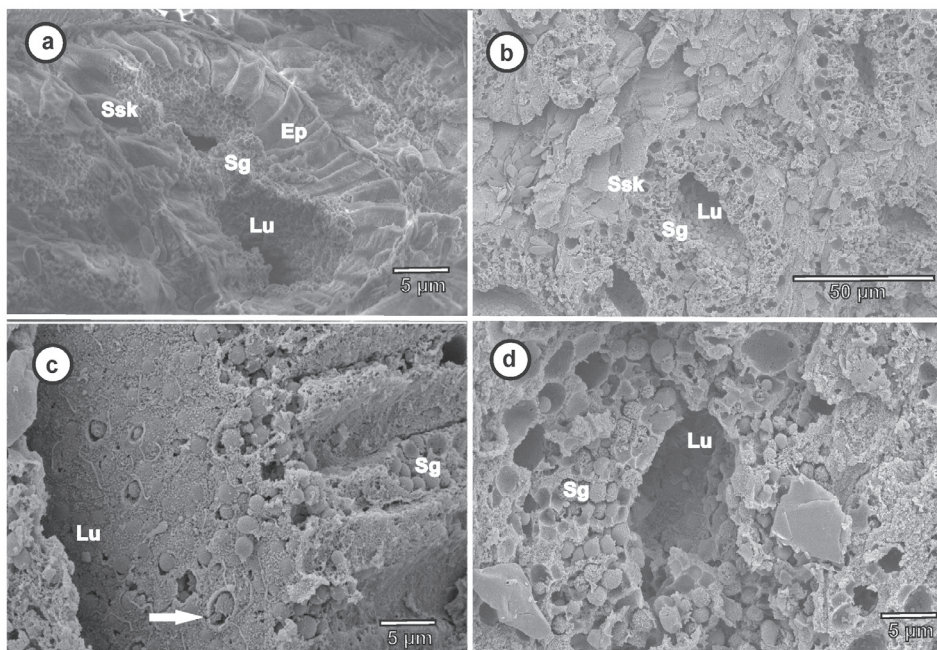
In analyzing the CDs, a modification was observed at the end of this tubule, which took on characteristics differing from those presented previously. Like the cells of the coating epithelium, they became higher, with a nucleus in the basal portion and presence of secretory granules in the apical portion. These were similar to the characteristics presented by the SSK (Figure 2). Thus, through morphological and histological analysis, the SSK of *N. frenata* and *A. dorsivittatum* was characterized as derived from a hypertrophied portion of the collecting tubules.

The SSK was observed in this study only in adult males of *N. frenata* and *A. dorsivittatum* and was not present in the young males analyzed. Using optical microscopy and staining with H&E, the

SSK was seen to have a coating on the tall columnar cells, with the nucleus in the basal portion of the cell (Figure 2). Lipid vacuoles were observed in the median portion of the SSK of both species (Figure 2b, d).

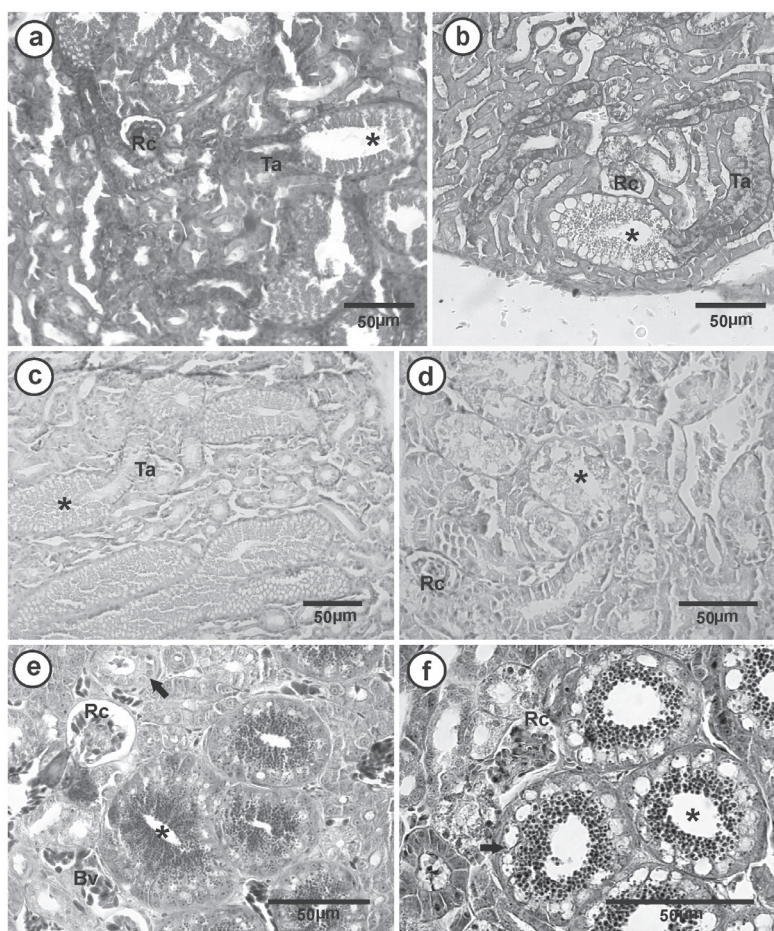
The scanning electron microscopy (SEM) analysis revealed that the SSK tubule was lined with simple columnar epithelium around a centralized lumen, with presence of secretory granules in the apical portion of the cell (Figure 3).

In the kidneys of the sexually mature males of *N. frenata* (Figure 4a) and *A. dorsivittatum* (Figure 4b) stained with PAS, the SSK epithelial cells did not present a positive reaction to PAS (Figure 4a, b). In some regions, a narrow-diameter tubular portion lined with a more flattened, although still columnar epithelium, was observed in the renal parenchyma. The cells of this epithelium presented mild-to-moderate PAS positivity in the apical portion, possibly associated with its secretory composition. In both species studied, transition areas between



**Figure 3** - Scanning electron microscopy kidney sections in *Notomabuya frenata* (a, c) and *Aspronema dorsivittatum* (b, d). Sexual segment of the kidney (Ssk); Lumen (Lu); Columnar epithelium (Ep); Secretory granules (Sg); Granules are secreted by epithelial cells (white arrow).





**Figure 4** - Light microscopy of kidney sections stained with periodic acid-Schiff (PAS) (a, b), Alcian Blue (AB) (c, d) and Gomori's trichrome (e, f) of *Notomabuya frenata* (a, c, e) and *Aspronema dorsivittatum* (b, d, f). Renal corpuscle (Rc); Sexual segment of the kidney (\*); Transition area (Ta); Blood vessel (Bv); Collagen fibers (black arrow).

CD and SSK stained positively with PAS (Figure 4a, b).

Alcian blue staining on the kidneys of *N. frenata* (Figure 4c) and *A. dorsivittatum* (Figure 4d) presented different reactions. The kidneys of *N. frenata* showed mild-to-moderate positivity in the apical portion of some tubules, which characterized the transition area between the CD and the SSK (Figure 4c). The SSK showed a negative reaction to AB in the beads and the vacuoles. Alcian blue staining also showed the transition area between the CD and SSK (Figure 4c). In *A. dorsivittatum*, there was a negative reaction to AB (Figure 4d).

Specimens of *N. frenata* (Figure 4e) and *A. dorsivittatum* (Figure 4f) showed renal parenchyma with a meager portion of connective tissue in the interstitium (as indicated by Gomori's trichrome), in which there were delicate bundles of collagen around the tubules and the renal parenchyma (Figure 4e, f).

## DISCUSSION

In the individuals of *N. frenata* and *A. dorsivittatum* analyzed in this study, presence of columnar epithelium of simple columnar type was observed, with tall cells, nucleus in the basal portion and

secretory granules in the medial and/or apical portion (Table I). These morpho-histological features have been observed in several families of Squamata and are summarized in Table I.

A probable mucoid secretion (as indicated by protein secretory granules) was observed in the SSK lumen of the individuals examined (Figure 2), thus forming a possible muco-proteinic secretion. The form of release of the exocrine secretion in the SSK is variable, and release in holocrine, merocrine and apocrine forms is possible. These forms have also been observed in other studies on the SSK (Table I) (Sanyal and Prasad 1966, Del Conte and Tamayo 1973, Sever and Hopkins 2005, Rheubert et al. 2011, 2014). Sever and Hopkins (2005) pointed out that SSK granules from *Scincella lateralis* (Say, 1823) (Scincidae) collected in North Carolina (United States) might contain a complex of lipids, glycogen, mucopolysaccharides and phosphatases; and that the chemical composition might change during the reproductive seasons of the year. This emphasizes the need for more detailed studies such as on histochemistry, to elucidate the secretory composition of SSK not only in *N. frenata* and *A. dorsivittatum*, but also in the Squamata group.

The SSK of the individuals analyzed in the present study on *N. frenata* and *A. dorsivittatum* did not react with PAS and Alcian blue, and was therefore negative for acid and neutral glycosaminoglycans. Mendes et al. (2009) also did not observe any positive PAS reaction in analysis on the SSK of *Tropidurus torquatus* (Wied, 1820) (Tropiduridae). However, these authors did not analyze the reaction to Alcian blue. Sever et al. (2012) tested the SSK of several snakes from different families and observed both PAS-negative and PAS-positive strains. Sever et al. (2012) observed a positive reaction to Alcian blue only in Homalopsidae. A positive reaction to Alcian blue was also observed in the SSK of *Hemidactylus turcicus* (Linnaeus, 1758) (Gekkonidae), in an analysis by Rheubert et al. (2011). However, in the majority of the species

studied, a negative reaction to Alcian blue was observed, thus indicating that absence of a positive reaction to Alcian blue has, until the present study, been a characteristic of this structure in Squamata. In *A. dorsivittatum*, neither the SSK nor the Alcian blue-positive transition areas were observed. This can possibly be explained by the difference in secretory composition in relation to the transition area of *N. frenata*. However, more detailed studies, such as on the histochemical components of both SSK and transitional area secretion in both species, would be required.

Del Conte and Tamayo (1973) reported that the first portion of the SSK of *Cnemidophorus lemniscatus* (Linnaeus, 1758) (Teiidae) had higher affinity for PAS than the second portion. This was because the second portion provides lower glycosaminoglycan secretion content and higher abundance of proteins than the first portion. The absence of positive PAS and Alcian blue findings in the SSK of *N. frenata* and *A. dorsivittatum* and the presence of positivity in the transition area may be related to the amount or even the presence/absence of mucopolysaccharides and proteins in the secretory granules of the SSK.

Few studies have investigated the reaction of the SSK to Gömöri's trichrome. The kidneys of *N. frenata* and *A. dorsivittatum* stained with Gomori's trichrome presented similar reactions around the structures of the renal parenchyma. This result was already expected since the renal tubules are arranged in interstitial tissue containing little connective tissue. Until the present study, the SSK in lizards was characterized as a hypertrophied region of three portions of the nephron (intermediate segment, distal convoluted tubule and collecting tubule) and of the ureter (Rheubert et al. 2014). Rheubert et al. (2011) studied the SSK of *Hemidactylus turcicus* (Linnaeus, 1758) (Gekkonidae) and presented two phylogenies that took into account the location of the SSK in Squamata, derived from morphological data (provided by Conrad 2008) and molecular data

**TABLE I**  
**Characteristics of the sexual segment of the kidney of Squamata reptiles already studied and those observed in**  
*Notomabuya frenata* and *Aspronema dorsivittatum* (present study).

Species*	Study Country	Reproductive Mode	Epithelium**	Nucleus***	Secretion****	Hypertrophied Portion*****	PAS	AB	Reference
LACERTILIA									
Teiidae									
<i>Cnemidophorus lemniscatus</i> (Linnaeus, 1758)	Venezuela	Oviparous	Columnar	Basal	Holocrine / Merocrine	CD	+	-	Del Conte and Tamayo 1973
Phrynosomatidae									
<i>Phrynosoma cornutum</i> (Harlan, 1824)	NE	Oviparous	Columnar	Basal	N/A	DCT	N/A	N/A	Anderson 1960
Lacertidae									
<i>Podarcis tauricus</i> (Pallas, 1814)	NE	Oviparous	Columnar	Basal	N/A	DCT/CD	N/A	N/A	Gabri 1983
Gekkonidae									
<i>Hemidactylus turcicus</i> (Linnaeus, 1758)	EUA	Oviparous	Columnar	Basal	Merocrine	CD	+	+	Rheubert et al. 2011
<i>Hemidactylus flaviviridis</i> Rüppell, 1835	India	Oviparous	Columnar	Basal	Apocrine	CD	+	-	Sanyal and Prasad 1966
Tropiduridae									
<i>Tropidurus torquatus</i> (Wied, 1820)	Brazil	Oviparous	Columnar	Basal	N/A	CD	-	N/A	Mendes et al. 2009
Scincidae									
<i>Scincella lateralis</i> (Say, 1823)	EUA	Oviparous	Columnar	Basal	Apocrine	DCT/CD	+	+	Sever and Hopkins 2005
Mabuyidae									
<i>Notomabuya frenata</i> (Cope, 1862)	Brazil	Viviparous	Columnar	Basal	N/A	CD	-	-	present study
<i>Aspronema dorsivittatum</i> (Cope, 1862)	Brazil	Viviparous	Columnar	Basal	N/A	CD	-	-	present study
SERPENTES									
Viperidae									
<i>Agkistrodon piscivorus</i> (Lacépède, 1789)	EUA	Viviparous	Columnar	Basal	Apocrine / Merocrine	PCT	+	+	Sever et al. 2008
Acrochordidae									
<i>Acrochordus granulatus</i> (Schneider, 1799)	Philippines	Viviparous	Columnar	Basal	N/A	DCT	-	-	Sever et al. 2012
Elapidae									
<i>Micrurus tener</i> Baird & Girard, 1853	EUA	Oviparous	Columnar	Basal	N/A	DCT	+	-	Sever et al. 2012



TABLE I (continuation)

Species*	Study Country	Reproductive Mode	Epithelium**	Nucleus***	Secretion****	Hypertrophied Portion*****	PAS	AB	Reference
<i>Naja samarensis</i> Peters, 1861	Philippines	Oviparous	Columnar	Basal	N/A	DCT	+	-	Sever et al. 2012
<i>Aipysurus eydouxii</i> (Gray, 1849)	Malaysia	Viviparous	Columnar	Basal	N/A	DCT	+	-	Sever et al. 2012
<i>Ephalophis greyae</i> Smith, 1931	Australia	Viviparous	Columnar	Basal	N/A	DCT	-	-	Sever et al. 2012
<i>Hydrophis lamberti</i> Smith, 1917	Philippines	Viviparous	Columnar	Basal	N/A	DCT	+	-	Sever et al. 2012
<i>Hydrophis ornatus</i> (Gray, 1842)	Malaysia	Viviparous	Columnar	Basal	N/A	DCT	+	-	Sever et al. 2012
<i>Hydrophis platurus</i> (Linnaeus, 1766)	Costa Rica	Viviparous	Columnar	Median	Apocrine	DCT	+	-	Sever et al. 2012
<i>Laticauda colubrina</i> (Schneider, 1799)	Philippines	Oviparous	Columnar	Basal	N/A	DCT	+	-	Sever et al. 2012
Homalopsidae									
<i>Cerberus rynchops</i> (Schneider, 1799)	Philippines	Viviparous	Columnar	Basal	N/A	DCT	+	+	Sever et al. 2012
Leptotyphlopidae									
<i>Rena dulcis</i> Baird & Girard, 1853	EUA	Oviparous	Columnar	Basal	N/A	DCT/CD	+	-	Sever et al. 2012
Uropeltidae									
<i>Teretrurus sanguineus</i> (Beddome, 1867)	India	Viviparous	Columnar	Basal	N/A	DCT	-	-	Sever et al. 2012
Colubridae									
<i>Sibynomorphus mikanii</i> (Schlegel, 1837)	N/E	Oviparous	N/E	Basal	N/A	N/E	N/A	N/A	Rojas and Almeida-Santos 2008
AMPHISBAENIA									
Amphisbaenidae									
<i>Amphisbaena vermicularis</i> Wagler, 1824	Brazil	Oviparous	Columnar	Basal	N/A	CD	N/A	N/A	Valverde et al. 2005
<i>Amphisbaena alba</i> Linnaeus, 1758	Brazil	Oviparous	Columnar	Basal	N/A	CD	N/A	N/A	Valverde et al. 2005
<i>Amphisbaena polystergum</i> (Duméril, 1851)	Brazil	Oviparous	Columnar	Basal	N/A	CD	N/A	N/A	Valverde et al. 2005
		Oviparous							

(NA) - not analyzed; (NE) - not specified; (CD) - collecting ducto; (DCT) - distal convoluted tubule; (PCT) - proximal convoluted tubule; (+) – PAS/AB positive; (-) – PAS/AB negative. \*Taxonomic classification according to Pyron et al. 2013. \*\*Epithelium lining the sexual segment of the kidney. \*\*\*Position of the nucleus in the cell lining epithelium of the sexual segment of the kidney. \*\*\*\*Figure secretion in epithelial cell lining of the sexual segment of the kidney. \*\*\*\*\*Hypertrophied portion of the kidney.

(provided by Eckstut et al. 2009). Conrad (2008) presented phylogeny based on morphological data on the head, such as bones and muscles of the skull, while the phylogeny provided by Eckstut et al. (2009) was derived from molecular data relating to the only one nuclear gene (*c-mos*). According to this study, the SSK of the Scincidae family would comprise hypertrophy of a portion of the collecting tubule and a portion of the ureter, both in morphological and in molecular analysis. Analysis on the two phylogenies revealed that different families shared the location of the SSK according to the characteristics analyzed. This demonstrates how variable the location of SSK is and, furthermore, the need for evolutionary studies relating to this structure in lizards.

Taxonomic studies are mainly based on external morphological characters (such as scales and coloration) and on molecular data (DNA). The difference observed in the external morphology of the kidneys of *N. frenata* (smooth texture) and *A. dorsivittatum* (granular texture) (Figure 1) indicates a characteristic of the species. In this way, when perceiving the differences in the internal morphology (organs), a complementary form of analysis for taxonomic studies is suggested. Another feature that can be used as taxonomic character is the location of SSK. *N. frenata* and *A. dorsivittatum* previously belonged to the Scincidae family; from the work of Hedges and Conn (2012), were reorganized in the new family Mabuyidae. The hypertrophied region of the nephron for Scincidae was a portion of the collecting tubule and ureter (Rheubert et al. 2011). In the present study, only a hypertrophy of the collecting tubule was observed. This fact differs between the two families; however, it becomes necessary to further study among the species that make up both families to define such a characteristic as a standard.

Several studies on the SSK in lizards (Sanyal and Prasad 1966, Del Conte and Tamayo 1973, Gabri 1983, Sever and Hopkins 2005, Mendes et

al. 2009, Rheubert et al. 2011, 2014) have shown a modified sex segment for which cyclical variation would be related to the interstitial cells of the testes. Thus, the activity and hypertrophy of the organ could be an androgen-dependent secondary sexual characteristic (Forbes 1941, Krohmer et al. 2004, Rheubert et al. 2014). However, studies detailing the immunohistochemistry, the consequent function of the secretion from the SSK and the mechanisms involved in its action are needed, both for the lizards in this study and for Squamata generally.

Studies on the SSK in lizards have been conducted more recently than those on this structure in snakes, and in comparison with most studies conducted on oviparous species (Table I). Regarding studies on viviparous species, SSK characterizations are almost nonexistent. Therefore, the present study adds to the knowledge of the reproductive biology and related structures of the reproductive strategies of two species of viviparous Neotropical lizards that hitherto had not been studied. This study has elucidating the morphological and histological characteristics of the SSK, which is a very important structure for understanding the reproductive biology of Squamata (reptiles).

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