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RESEARCH ARTICLE

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Male genital system of *Ameiva ameiva* (Squamata: Teiidae)

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Abstract
Understanding squamate reproductive morphology is crucial for investigating ecological, behavioral, and evolutionary questions. Here, we describe the anatomy and histology of the male genital system of *Ameiva ameiva* from southeastern Brazil. Ten adult males were dissected to characterize genital macroscopy and collect fragments of the testes, gonadoducts, and kidneys for histological examination. We examined 10 transverse histological sections per individual and measured the epithelial height of the epididymis and ductus deferens. The male reproductive system consists of a pair of yellowish oval testes, the rete testis, ductuli efferentes, epididymis, ductus deferens, ampulla ductus deferentis, sexual segment of the kidney (SSK), cloaca, and hemipenis. The hemipenis is elongated, cylindrical, and unilobed, with a sulcate face and an asulcate face, which has continuous fringes throughout its length. Seminiferous tubules exhibited germ cells at various stages. The epididymis is wider and more coiled than the ductus deferens. The rete testis has a simple squamous epithelium with long stereocilia, while the narrower ductuli efferentes are lined by a simple ciliated cuboidal epithelium. The epididymal epithelium is pseudostratified columnar, with basal and ciliated principal cells, whereas the ductus deferens epithelium is pseudostratified to simple cuboidal. The epididymal epithelium is 1.5 times taller than the ductus deferens epithelium. Here, we observed the SSK present in the cortex of the ventral region of the kidneys due to the hypertrophy of the distal convoluted tubules, as well as its secretory activity. Our findings will contribute to future research into the evolution of squamate reproductive morphology.

KEYWORDS
ductus deferens, epididymis, hemipenis, reproductive morphology, rete testis, sexual segment of the kidney, testis

1 | INTRODUCTION

Understanding animal reproductive morphology is crucial because it lays the groundwork for addressing ecological, behavioral, and evolutionary questions. For example, by examining the gross morphology of male reproductive organs, we can gain insights into the evolution of mating systems and sperm competition (e.g., Lüpold et al., 2009). Additionally, knowledge of reproductive anatomy can help clarify evolutionary relationships by identifying useful morphological traits (e.g., Siegel et al., 2011). Therefore, research on reproductive morphology plays a fundamental role in advancing our understanding of animal biology, ecology, and evolution.

In squamates, the male reproductive system consists of testes, gonadoducts, the sexual segment of the kidneys

(SSKs), the cloaca, and the hemipenis (Rheubert et al., 2014; Trauth & Sever, 2011). In lizards, the testes are paired, ovoid, and compact organs surrounded by the tunica albuginea; they are located within the coelomic cavity and attached to the body wall by the mesorchium (Gribbins & Rheubert, 2014). Testicular coloration and size vary due to anatomical asymmetries and reproductive seasonality (Gribbins & Rheubert, 2014). Histologically, the testicular parenchyma consists of germ line cells (which are supported by Sertoli cells), whereas the testicular interstitium includes Leydig cells, fibroblasts, collagen fibers, blood vessels, lymphatic vessels, and nerves (Colli & Pinho, 1997; Gribbins, 2011; Gribbins & Rheubert, 2014).

Sperm are produced in the seminiferous tubules within the testes and are then sequentially transported through the gonadoducts—the rete testis, ductuli efferentes, epididymis, and ductus deferens—before finally reaching the cloaca (Gribbins, 2011; Rheubert et al., 2014). The rete testis is the least studied region of the squamate gonadoduct (Rheubert et al., 2014). It consists of either a single tubule or a network of interconnected tubules that collect spermatozoa produced by the seminiferous tubules and transport them to the ductuli efferentes (Rheubert et al., 2014, 2020). The ductuli efferentes are narrower than the rete testis and consist of a series of tubules lined by a simple ciliated cuboidal epithelium, connecting the rete testis to the epididymis (Rheubert et al., 2020). The epididymis, which is lined by pseudostratified columnar epithelium, is the most coiled region of lizard gonadoducts (Rheubert et al., 2014, 2020). The ductus deferens is wider than the epididymis, and in some species, it ends in a single duct known as the ampulla ductus deferentis (Pewhom & Srakaew, 2018; Rheubert et al., 2014). The ductus deferens in lizards has also received limited microscopic morphological investigation (Rheubert et al., 2014). The hemipenes are paired copulatory organs located within the tail, posterior to the cloaca (Hildebrand et al., 2006; Knotek et al., 2017). During copulation, they are everted and used one at a time (Halliday, 1994). In many lineages, these organs display ornamentation such as spines, calyxes, papillae, fringes, and pouches to facilitate copulatory coupling (Nunes, 2011). Hemipenes are valuable tools in systematic and taxonomic studies, used to differentiate genera and species (Harvey et al., 2012; Nunes et al., 2012; Silva et al., 2013).

A distinct feature of squamates is that their kidneys also have a reproductive function. Certain parts of the renal parenchyma undergo hypertrophy and epithelial modifications to form the SSK (Aldridge et al., 2011; Migliore et al., 2024; Rheubert et al., 2014). The SSK cells produce secretory granules that mix with the spermatozoa, providing them with nutrition (Aldridge et al., 2011; Rheubert et al., 2014; Sever & Hopkins, 2005). The SSK is testosterone-dependent and related to the activity of testicular interstitial cells (Mendes et al., 2009; Novelli et al., 2018; Rojas et al., 2013).

Although research on squamate reproductive biology has considerably advanced in the last decades, there is still a lack of studies examining the male reproductive anatomy (reviewed in Aldridge et al., 2011; Gribbins & Rheubert, 2011, 2014; Trauth & Sever, 2011; Rheubert et al., 2014). Available studies indicate that certain regions of the male reproductive system in lizards are evolutionarily conserved, while others vary considerably across lineages (Gribbins & Rheubert, 2014; Rheubert et al., 2014, 2020). Therefore, additional research on reproductive morphology in various lizard lineages is necessary to fully understand the diversity of reproductive morphology in this group.

Here, we focus on the male reproductive morphology of the teiid lizard *Ameiva ameiva* (Linnaeus, 1758), a widespread species in tropical and subtropical South America (Vitt et al., 2008). In Brazil, *A. ameiva* is found in the Amazon, Caatinga, and Cerrado biomes, and is particularly abundant in São Paulo state (Araujo & Almeida-Santos, 2011; Guedes et al., 2023). According to Rocha (2008) and Vitt & Colli (1994), this species has greater reproductive activity between spring and summer seasons. Therefore, our study specifically aims to describe the macro and microscopic anatomy of the male genital system of *A. ameiva* from southeastern Brazil.

2 | MATERIALS AND METHODS

2.1 | Specimen collection

We collected 10 adult male *A. ameiva* by active search between June 2017 and March 2018 in a rural area of Olímpia municipality (20° 44′ 13″ S, 48° 54′ 54″ W; 506 m altitude), São Paulo state, Southeastern Brazil (collection permit: 59560-1/SISBIO). The lizards were transported to the Laboratory of Comparative Anatomy at the Instituto Federal de São Paulo (Barretos municipality), where they were kept at room temperature (27.0 ± 0.5°C) and under a natural photoperiod (∼14 h light, 10 h dark) for 5 days to allow for acclimatization (Clusella-Trullas & Chown, 2014). During this period, the lizards were fed fruits and earthworms ad libitum (Kahrl & Cox, 2015).

2.2 | Data collection

The lizards were anesthetized by isoflurane inhalation (1 mL/mL) and then euthanized by injecting potassium chloride (75 mg/kg) into the heart (CFMV, 2012). A midventral incision was made from the scapular girdle to the cloaca to expose the reproductive organs. Photodocumentation was conducted using a Leica DFC295 digital camera mounted on a Leica MZ16 stereomicroscope. Next, the left urogenital organs were excised for histological analysis. The animal handling and experimental procedures were approved by the Ethics and Animal Experimentation Committee of the São Paulo State University (approval number 177/2017).

2.3 | Histological processing and analysis

The excised organs were fixed in a methacarn solution (60% methanol, 30% chloroform, and 10% glacial acetic acid) for 4 h (Howat & Wilson, 2014). Next, the samples were washed in running water, dehydrated using a series of increasing ethanol concentrations (70%–100%), and then embedded in paraffin (Paraplast, Sigma). The left testis, epididymis, ductus deferens, and kidney were processed for standard histological analysis.

For histometric analyses, we obtained 10 histological cross sections (each 5 μm thickness) from each organ of all collected animals. The sections were stained with hematoxylin–eosin (H-E) to describe sperm production and epithelial cells, as well as to measure the epithelial height of the epididymis and ductus deferens. The periodic acid-Schiff (PAS) reaction was performed to detect glycoproteins in the testis, epididymis, ductus deferens, and kidney. Gomori's trichrome and picrosirius red were used to identify collagen and smooth muscle fibers in the testis, kidney, and interductal region of the epididymis and ductus deferens. Photodocumentation was conducted using a Leica DM 4000B light microscope equipped with a Leica DFC 280 image capture system and Image Pro-Plus software (Media Cybernetics Inc.). For each of the 10 animals, we randomly analyzed 10 transverse histological sections of the epididymis and ductus deferens. We then measured four epithelial cells on diametrically opposite sides of the same duct, resulting in 40 measurements per animal.

2.4 | Statistical analysis

Student's *t*-test was performed to compare the height of the epididymis and the ductus deferens. We used the R software version 3.3.2—Development Core Team, 2016. To attribute statistical signification, the *p* value was set at $p \leq 0.05$ (Zar, 1999).

3 | RESULTS

The male genital system of *A. ameiva* varies within and among individuals, especially in terms of the shape, size, and positioning of the testes, as well as the height of the gonadoduct epithelium.

The partially everted hemipenes are unilobed, elongated, and cylindrical (Figure 1a,b). The sulcate face of the hemipenis has a deep spermatic groove that extends throughout the organ (Figure 1b), while the asulcate face has continuous fringes throughout its contour (Figure 1a).

The testes are paired and positioned asymmetrically, with the right testis located more cranially than the left (Figure 2a). They are located in the posterior portion of the abdominothoracic cavity, between the ventral side of the kidneys and the intestinal loops (Figures 1c and 2a). The testes are oval and yellowish, with blood vessels visible through the transparent tunica albuginea (Figure 2a,b). Histologically, the testicular parenchyma is composed of seminiferous tubules (Figure 3). Within these tubules, there is a germinal epithelium consisting of Sertoli cells located near the basal lamina, as well as germ cells at various stages of differentiation (Figure 3a). Spermatogonia, which are located closer to the basal lamina, stain more intensely with hematoxylin (Figure 3a). Primary spermatocytes, which are larger than spermatogonia, undergo meiotic division and generate smaller secondary spermatocytes. These secondary spermatocytes then develop into spermatids, initially having rounded shapes and highly condensed chromatin. As the spermatids differentiate, they elongate and eventually transform into spermatozoa. Spermatozoa are positioned centrally within the tubules and are released into the lumen (Figure 3a).

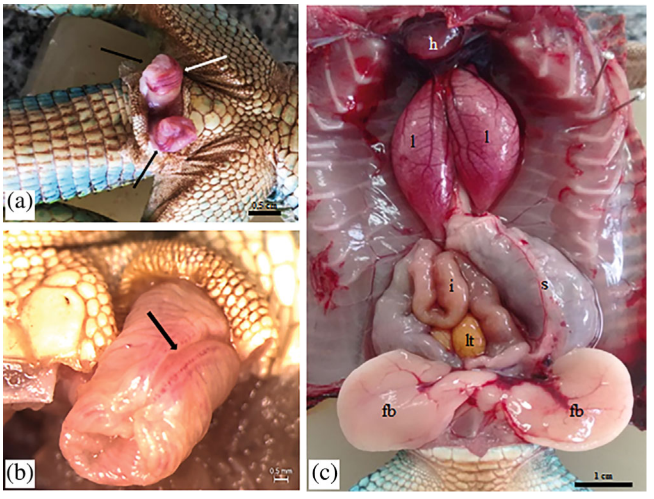

FIGURE 1 Gross morphology of the hemipenes and body cavity of *A. ameiva*. (a) Pair of hemipenes partially everted, showing the sulcate face with spermatic groove (black arrows) and the asulcate face with fringes (white arrow). (b) Detail of the spermatic groove (black arrow). (c) Abdominal cavity showing the heart (h), lungs (l), stomach (s), intestine (i), left testis (lt), and fat bodies (fb).

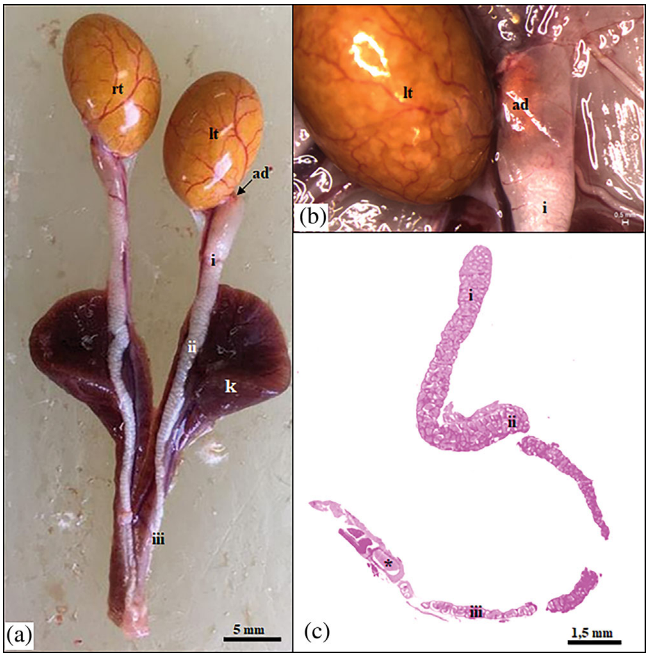

FIGURE 2 General view of the male urogenital system of *A. ameiva*. (a) Gross morphology of the right (rl) and left testis (lt), adrenal gland (ad), and the seminiferous pathway: epididymis (i); proximal (ii) and distal (iii) portions of the ductus deferens in syntopia with the ventral surface of the kidneys (k). (b) Detail of the adrenal gland (ad) attached to the epididymal sheath, between the left testis (lt) and the epididymis (i). (c) Histological overview of the left seminiferous pathway (H-E), showing epididymis (i), ductus deferens (i and ii) and ampulla (*). The ductus deferens curved during the paraffin embedding process.

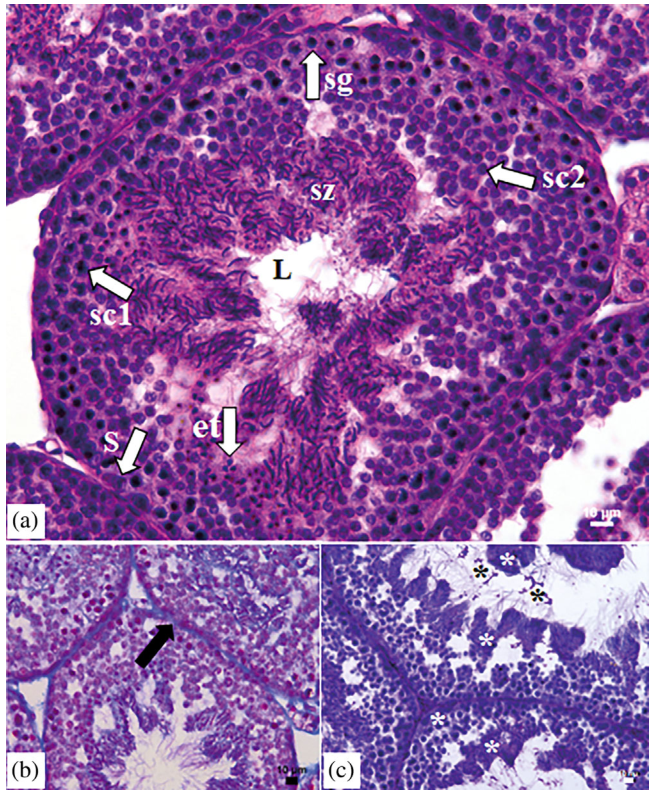

FIGURE 3 Cross sections of the testis of *A. ameiva*. (a) Seminiferous tubules with Sertoli cells (S) and germ cells: spermatogonia (sg), primary spermatocytes (sc1), secondary spermatocytes (sc2), round spermatids (et) and sperm bundles (sz) released into the lumen (L) (H-E staining). (b) Seminiferous tubules with collagen fibers around the tubules (black arrow), stained with Gomori's trichomic. (c) Seminiferous tubule showing the cytoplasm of Sertoli cells containing glycoproteins intensely stained with PAS (*).

The interstitial region contains collagen fibers that stain intensely with Gomori's trichrome (Figure 3b). Sertoli cells elongate and extend from the basal region of the seminiferous tubules to the lumen; the elongations are rich in glycoproteins, as evidenced by the positive reaction to PAS (Figure 3c).

The adrenal glands are small, yellowish, globular structures located dorsocaudally to the testes and anteriorly to the spermatic ducts (Figure 2a,b). They are closely attached to the testes by the epididymal sheath, as are the anterior testicular ducts (Figure 2b).

The rete testis and ductuli efferentes are attached to the epididymal sheath (Figures 2b and 4a) and are located near the adrenal glands. They begin as a set of small tubules emanating from the testes and form the rete testis (Figure 4a). The rete testis has a simple squamous epithelium with long stereocilia and a large lumen (Figure 4b). The ductuli efferentes have a simple cuboidal epithelium composed of ciliated cells with central and voluminous nuclei (Figure 4c). The lumen of the ductuli efferentes also contains sperm, but its diameter is much smaller than that of the rete testis (Figure 4a,c).

The epididymal ducts originate from the ductuli efferentes (Figure 4a). The epididymides are tightly coiled tubular structures that extend to the cranial margin of the kidneys (Figure 2a). The epididymal epithelium is tall columnar pseudostratified (mean = 25.177 ± 5.979 μm; Figures 2c and 5a–d). The epididymal epithelium consists of basal cells and ciliated principal cells (Figure 4d). Numerous spermatozoa are present in the epididymal lumen (Figures 4d and 5a,f).

The ductus deferens primarily occupies the medial and distal portions of the gonadoduct, adjacent to the entire ventral region of the kidneys (Figure 2a). The ductus deferens is less coiled and thinner than the epididymis (Figures 2c and 5b,c). The epithelium of the ductus deferens is pseudostratified, changing to a simple cuboidal epithelium (mean = 14.940 ± 3.468 μm) along its length (Figure 5b,c,g). Moreover, the epithelium of the ductus deferens exhibits increased interductal smooth muscle and conspicuous adjacent connective tissue

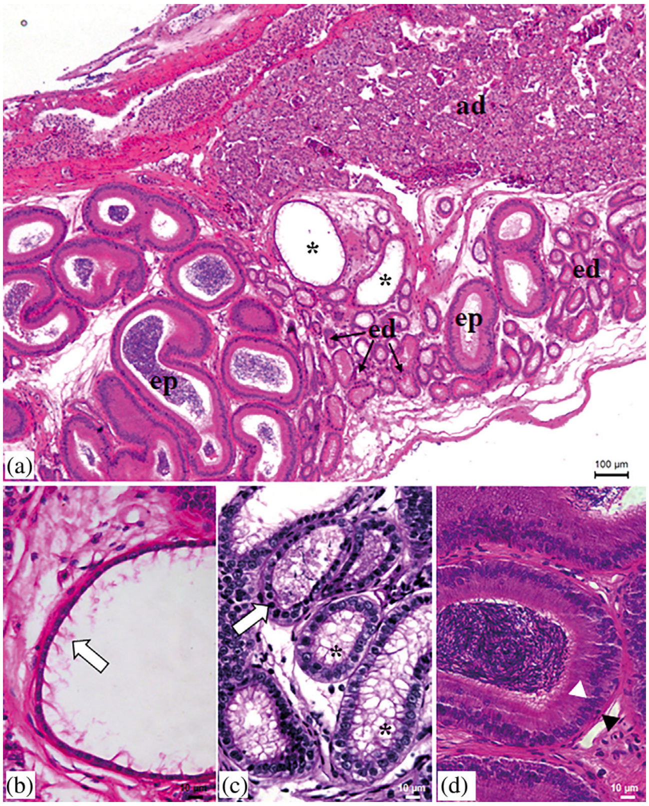

FIGURE 4 Histological sections of the initial part of the seminiferous pathway of *A. ameiva* (H-E staining). (a) Adrenal gland (ad), rete testis (*), ductuli efferentes (ed), and epididymal ducts (ep). (b) Detail of the rete testis showing a simple squamous epithelium with stereocilia (arrow). (c) Ductuli efferentes with a simple cubic epithelium, cells with a central and voluminous nucleus (arrow), and many cilia in the lumen of the duct (*). (d) Detail of the epididymis, showing pseudostratified columnar epithelium with ciliated cylindrical cells (white arrowhead) and cells with basal nuclei (black arrowhead).

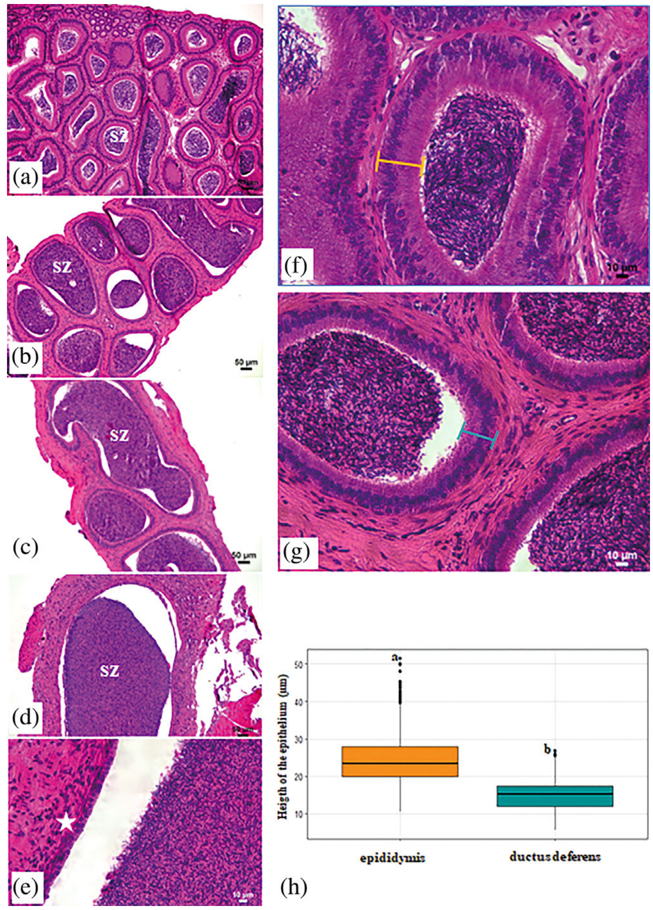

FIGURE 5 Histological cross sections of the seminiferous pathway of *A. ameiva* (H-E staining). Epididymis (a), ductus deferens (b and c) and ampulla ductus deferentis (d). (e) Ampulla epithelium formed by simple and cuboidal cells (black arrow), with adjacent tissue formed by smooth muscles (star) and connective tissue (*). Morphological (f and g) and statistical (h) differences ($p < 0.05$) between the height of the epididymal epithelium (yellow bar) and the ductus deferens epithelium (green bar), different letters mean statistical differences. The box represents the first and third quartiles. The lines inside the box represent the median, and the points represent the outliers. *, connective tissue; sz, spermatozoa.

(Figures 5b,c,g and 6c). The ductus deferens epithelium is also lower than the epididymal epithelium ($t = -35.726$, df $= 303.35$, $p < 2.2e\text{-}16$; Figures 5f,g,h) and reacted positively for PAS in three out of four individuals. The distal ductus deferens enlarges to form the ampulla ductus deferentis, which is surrounded by abundant connective tissue and contains bundles of smooth muscle adjacent to the epithelium (Figures 2c and 5d,e). The ampulla ductus deferentis consists of simple cuboidal epithelial cells (Figure 5e).

The amount of collagen fibers varies along the seminiferous pathway. In the testes, collagen fibers appear as a thin layer in the interstitial region (Figure 6a). The abundance of collagen fibers is lower in the epididymides (Figure 6b) but increases as the degree of coiling and epithelial thickness of the ductus deferentis decreases (Figure 6c).

The SSK is located in the ventral cortex of the kidney and is formed through the differentiation and hypertrophy of the distal convoluted tubules, which also contain collagen fibers in the peritubular region (Figure 7a). The renal parenchyma exhibits a transition zone where the SSK forms from the hypertrophy of the distal convoluted tubules. This transition involves a shift from a simple cuboidal epithelium to a simple columnar epithelium (Figure 7b). The apical portions of the columnar epithelium react positively to PAS, indicating secretory function (Figure 7c).

4 | DISCUSSION

This study describes the macro- and microscopic morphology of the genital system of the lizard *A. ameiva*. We address the main morphological and histological distinctions

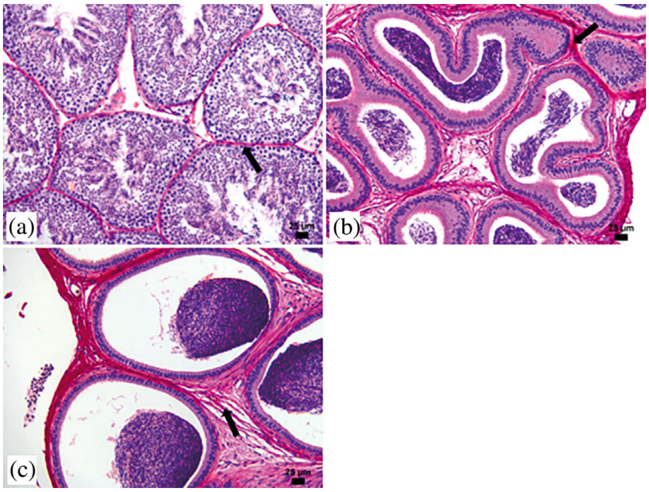

FIGURE 6 Histological cross sections of the testis (a), epididymis (b), and ductus deferens (c) of *A. ameiva* showing type I collagen fibers (black arrows) in the connective tissue of these organs (picrosirius red staining).

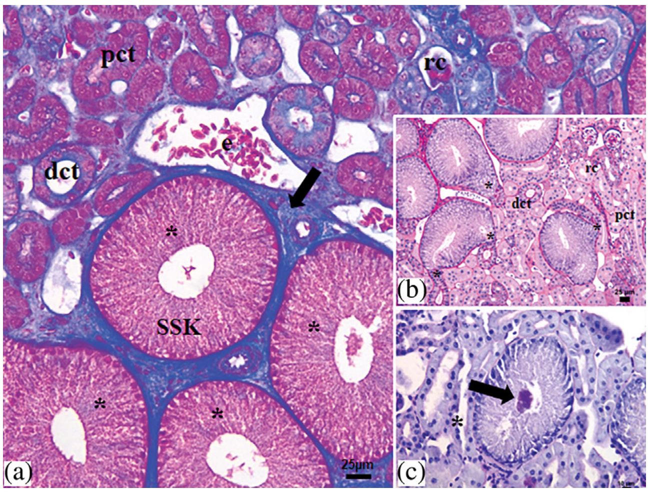

FIGURE 7 Histology of the kidney of *A. ameiva* highlighting the region containing the SSK. (a) Renal cortex showing proximal convoluted tubules (pct), distal convoluted tubules (dct), renal corpuscle (rc), erythrocytes within the blood vessel (e), and collagen fibers around the tubules (black arrow) (Gomori's trichomic staining). SSK with simple and high columnar epithelium (*). (b) SSK transition areas (*) showing renal corpuscles (rc), proximal convoluted tubules (pct), distal convoluted tubules (dct), and type I collagen fibers around the tubules (picrosirius red staining). (c) Secretory activity in the apical portion of the SSK cells (black arrow), marked by the positive reaction to PAS staining.

between the testicular and seminiferous structures, including the rete testis, ductuli efferentes, epididymis, ductus deferens, ampulla ductus deferentis, and SSK.

The gross morphology of the urogenital tract of *A. ameiva* is similar to that of other lizards. In lizards, the testes are paired, with the right testis slightly more cranial than the left testis (Rheubert et al., 2014). *A. ameiva* also exhibits this pattern. In most lizards, the testes are round to slightly oval and white to cream (Gribbins & Rheubert, 2014). In *A. ameiva*, the testes are ovoid and yellowish, as described for another teiid species (*Aspidoscelis sexlineatus viridis*: Trauth, 2020). The adrenal glands of squamates are yellowish or reddish and are located in the epididymal sheath (Rheubert et al., 2020; Sever, 2010). In *A. ameiva*, the adrenal glands are yellowish and located dorsocaudally to the testes, attached to the epididymal sheath. In *A. ameiva*, the rete testis and ductuli efferentes anastomose to form other regions of the gonadoducts, as in other lizards (Akbarsha et al., 2006; Gribbins, 2011; Pewhom & Srakaew, 2018; Rheubert et al., 2020; Sever, 2010). The epididymis and ductus deferens of *A. ameiva* are cylindrical and convoluted, as described in other lizards (Rheubert et al., 2014). However, the epididymis is more convoluted, and its epithelium is 1.5 times higher than that of the ductus deferens. Therefore, the gross anatomy of the urogenital system of *A. ameiva* reflects the highly conserved pattern of squamates. Similarly, the hemipenes of *A. ameiva* in this study closely resemble those described for other populations of this species (Nunes, 2011).

The testicular parenchyma of *A. ameiva* exhibits the same organization as higher vertebrates, in which spermatogenesis occurs in a wave-like pattern because of the progressive and synchronized development of germ cells along the seminiferous tubules (Ferreira et al., 2009; Yoshida, 2016). We identified all the cells of the germ line, which undergo progressive compaction of the chromatin during spermatogenesis. This compaction is indicated by the more intense staining with hematoxylin (Villagrán-SantaCruz et al., 2014). Moreover, the testicular epithelium stains intensely with PAS, indicating the presence of glycoproteins. These substances are secretion granules associated with the cytoplasm of Sertoli cells (Zieri et al., 2008). These granules nourish the germ line cells during spermatogenesis (Ferreira & Dolder, 2003); a condition common to all other vertebrates (Yoshida, 2016).

The rete testis in *A. ameiva* has a simple squamous epithelium. This pattern is recurrent in lizards from various families, such as gekkonids (*Hemidactylus turcicus*: Rheubert et al., 2010), phrynosomatids (*Sceloporus consobrinus* and *Sceloporus undulatus*: Rheubert et al., 2014, 2020), scincids (*Scincella lateralis*: Sever et al., 2013), agamids (*Leiolepis ocellata*: Pewhom & Srakaew, 2018), and anolids (*Anolis carolinensis*: Rheubert et al., 2020). The epithelial cells of the rete testis in *A. ameiva* have long stereocilia. Ciliated cells are also found in the rete testis of various lizards, including *H. turcicus* (Rheubert et al., 2010), *S. consobrinus* (Rheubert et al., 2014), and *L. ocellata* (Pewhom & Srakaew, 2018), but they are absent in *S. lateralis* (Sever et al., 2013). Therefore, the microscopic morphology of the rete testis seems to vary little in lizards (Rheubert et al., 2014).

The ductuli efferentes of *A. ameiva* have a simple cuboidal epithelium with numerous ciliated cells, a pattern that has been observed in many other lizards (Rheubert et al., 2010, 2014, 2020; Sever et al., 2013; Sharath et al., 2022). However, some variation seems to occur in agamids; the ductuli efferentes epithelium is ciliated columnar in *Sitana ponticeriana* (Akbarsha et al., 2007) and pseudostratified ciliated columnar to simple ciliated cuboidal in *L. ocellata* (Pewhom & Srakaew, 2018). The ductuli efferentes are hypothesized to serve various functions, such as the uptake of luminal fluid, mixing of seminal fluid, propulsion of spermatozoa to distal parts of the gonadoducts, and spermiophagy (Akbarsha et al., 2007; Rheubert et al., 2014; Sharath et al., 2022).

The epididymal epithelium of *A. ameiva* is pseudostratified columnar, as in many lizards (Rheubert et al., 2010, 2014, 2020; Pewhom & Srakaew, 2018). Basal cells and ciliated principal cells characterize the epididymal epithelium of lizards from various lineages, including Lacertidae, Tropiduridae, Gekkonidae, Anolidae, and Phrynosomatidae (Rheubert et al., 2014, 2020). Multiple cell types have been found only in Agamidae and Scincidae (Aranha et al., 2023; Rheubert et al., 2014). In *A. ameiva*, we only observed basal cells and ciliated principal cells. The epididymal epithelium in lizards secretes cellular products into the lumen. Although the function of these secretions remains unknown, they are likely involved in sperm maturation (Rheubert et al., 2014).

The epithelium of the ductus deferens in *A. ameiva* is also pseudostratified ciliated columnar. This epithelial type is also found in the ductal epithelium of other teiids (*Aspidoscelis gularis*: Rheubert et al., 2014 and *A. sexlineatus*: Trauth, 2020). Pseudostratified columnar epithelium also characterizes the ductus deferens in lizards from various lineages, such as agamids (Pewhom & Srakaew, 2018), scincids (Rheubert et al., 2014; Trauth, 2018), anolids, and phrynosomatids (Rheubert et al., 2020). In the varanid *Varanus niloticus*, the ductus deferens is lined with nonciliated simple columnar cells (Awad et al., 2021). The ductus deferens of *A. ameiva* is less coiled than that of *Calotes versicolor* (Akbarsha & Meeran, 1995) and has a relatively larger peritubular space (delineated by a higher number of collagen and muscle fibers) compared with *Varanus niloticus* (Awad et al., 2021).

The ampulla ductus deferentis has also been found in another teiid (*A. sexlineatus*: Trauth, 2020), one gekkonid (Gautreaux, 2016), three agamids (Akbarsha & Meeran, 1995; Akbarsha et al., 2005; Pewhom & Srakaew, 2018), four phrynosomatids (Rheubert et al., 2014), and two scincids (Rheubert et al., 2014; Trauth, 2018). However, the ampulla ductus deferentis is absent in the scincid *Plestiodon fasciatus* (Rheubert et al., 2014). Further studies are needed to better characterize the phylogenetic distribution of the ampulla ductus deferentis in lizards. In *A. ameiva*, the epithelium of the ampulla ductus deferentis changes from pseudostratified columnar in the ductus deferens to simple cuboidal. Moreover, in *A. ameiva*, the ampulla ductus deferentis is formed by a single duct and can only be identified through histological analyses, unlike the agamid *Sitana ponticeriana*, in which the ampulla can be observed macroscopically (Akbarsha et al., 2005). Around this duct, there is an increase in adjacent connective tissue and smooth muscle, as observed in the lizards *Calotes versicolor* (Akbarsha & Meeran, 1995) and *Sitana ponticeriana* (Akbarsha et al., 2005), and the snake *Agkistrodon piscivorus* (Siegel et al., 2009), suggesting a common trait in squamates. The role of the ampulla ductus deferentis includes sperm storage and contribution to seminal plasma by secreting and phagocytosing sperm (Akbarsha et al., 2005; Akbarsha & Meeran, 1995).

The SSK plays an important reproductive function in squamates. It is formed by the hypertrophy and modification of the epithelium in certain regions of the nephron, influenced by testosterone (Aldridge et al., 2011; Rheubert et al., 2014). In teiid lizards, the SSK is formed by hypertrophy of the distal convoluted tubules, collecting ducts, ureters, or all of these regions of the nephron (Rheubert et al., 2014). In *A. ameiva*, the cortex of the ventral region of the kidneys contacts the ductus deferens. In this region, only the distal convoluted tubules hypertrophy, changing from a simple cuboidal epithelium to a tall columnar epithelium with basal nuclei, thus forming the SSK. In the tropidurid lizard *Tropidurus torquatus* and the scincids *Notomabuya frenata* and *Aspronema dorsivitatum*, the SSK is formed from the collecting ducts of the nephron (Mendes et al., 2009; Migliore et al., 2024; Novelli et al., 2018). However, in the *anolid A. carolinensis* and the phrynosomatid *S. undulatus*, the SSK is formed from the hypertrophy of the distal convoluted tubules, the collecting ducts, and the ureters (Rheubert et al., 2020). Collectively, these observations corroborate that this structure varies widely across lizard families. SSK secretions may help maintain the viability of sperm stored in the ductus deferens, as observed in the lizards *Notomabuya frenata* (Migliore et al., 2024), *A. carolinensis* and *S. undulatus* (Rheubert et al., 2020), as well as the snakes *Philodryas patagoniensis* (Loebens et al., 2017) and *Dipsas mikanii* (Rojas et al., 2013). As in other squamates, the SSK of *A. ameiva* also secretes glycoprotein substances.

5 | CONCLUSION

We present a comprehensive morphological description of the genital system of the lizard *A. ameiva*, a widespread species in Brazil. We characterize the morphology of the testes and gonadoducts and describe the main differences among the structures that comprise this system. We also show the presence of the SSK, a structure unique to squamates, and its secretory activity. Given the limited research on the reproductive morphology of Neotropical lizards, particularly in Teiidae, we expect that our findings will contribute to future studies on the reproductive biology of squamates and create opportunities for further research with Neotropical lizard species.

AUTHOR CONTRIBUTIONS

Érica da Silva Maciel: Investigation; conceptualization; methodology; writing – original draft; funding acquisition; visualization; software; data curation. **Rodrigo Zieri:** Writing – review and editing; investigation; software; formal analysis; project administration; supervision; methodology; validation; data curation; writing – original draft; visualization. **Selma Maria de Almeida-Santos:** Funding acquisition; resources; supervision; data curation; formal analysis; project administration; writing – review and editing; validation; visualization.

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REFERENCES

Akbarsha, M. A., Kadalmani, B., & Tamilarasan, V. (2006). Histological variation along and ultrastructural organization of the epithelium of the ductus epididymidis of the fan-throated lizard *Sitana ponticeriana* Cuvier. *Acta Zoologica*, *87*(3), 181–196. https://doi.org/10.1111/j.1463-6395.2006.00231.x

Akbarsha, M. A., Kadalmani, B., & Tamilarasan, V. (2007). Efferent ductules of the fan-throated lizard *Sitana ponticeriana* Cuvier: Light and transmission electron microscopy study. *Acta Zoologica*, *88*, 265–274. https://doi.org/10.1111/j.1463-6395.2007.00278.x

Akbarsha, M. A., & Meeran, M. M. (1995). Occurrence of ampulla in the ductus deferens of the Indian garden lizard *Calotes versicolor* Daudin. *Journal of Morphology*, *225*(3), 261–268. https://doi.org/10.1002/jmor.1052250303

Akbarsha, M. A., Tamilarasan, V., Kadalmani, B., & Daisy, P. (2005). Ultrastructural evidence for secretion from the epithelium of ampulla ductus deferentis of the fan-throated lizard *Sitana ponticeriana* Cuvier. *Journal of Morphology*, *266*(1), 94–111. https://doi.org/10.1002/jmor.10369

Aldridge, R. D., Jellen, B. C., Siegel, D. S., & Wisniewski, S. S. (2011). The sexual segment of the kidney. In R. D. Aldridge & D. M. Sever (Eds.), *Reproductive biology and phylogeny of snakes* (pp. 478–510). Science Publishers.

Aranha, I., Bhagya, M., & Sagar, B. C. (2023). Epididymis of the lizard *Eutropis carinata*: A light microscopic and ultrastructural seasonal study. *Journal of Endocrinology and Reproduction*, *27*(1), 54–71.

Araujo, C. D. O., & Almeida-Santos, S. M. D. (2011). Herpetofauna de um remanescente de cerrado no estado de São Paulo, sudeste do Brasil. *Biota Neotropica*, *11*, 47–62.

Awad, M., Alshehri, M., & Hassaneen, A. S. A. (2021). Histological, histochemical, and ultrastructural approach to the ductus deferens in male nile monitor lizard (*Varanus niloticus*). *Microscopy and Microanalysis*, *27*(4), 935–940. https://doi.org/10.1017/S1431927621012046

CFMV. (2012). *Guia brasileiro de boas práticas a eutanásia em animais*. Conselho Federal de Medicina Veterinária.

Clusella-Trullas, S., & Chown, S. L. (2014). Lizard thermal trait variation at multiple scales: A review. *Journal of Comparative Physiology B*, *184*, 5–21. https://doi.org/10.1007/s00360-013-0776-x

Colli, G. R., & Pinho, A. A. (1997). Interstitial cell cycle of *Ameiva ameiva* (Sauria, Teiidae) in the Cerrado region of Central Brazil. *Journal of Morphology*, *233*(2), 99–104. https://doi.org/10.1002/(SICI)1097-4687(199708)233:2%3C99::AID-JMOR1%3E3.0.CO;2-J

Ferreira, A., & Dolder, H. (2003). Cytochemical study of spermiogenesis and mature spermatozoa in the lizard *Tropidurus itambere* (Reptilia, Squamata). *Acta Histochemica*, *105*(4), 339–352. https://doi.org/10.1078/0065-1281-00731

Ferreira, A., Silva, D. N., Van Sluys, M., & Dolder, H. (2009). Seasonal changes in testicular and epididymal histology of the tropical lizard, *Tropidurus itambere* (Rodrigues, 1987), during its reproductive cycle. *Brazilian Journal of Biology*, *69*, 429–435. https://doi.org/10.1590/S1519-69842009000200028

Gautreaux, J. C. (2016). *Urogenital morphology of the Mediterranean gecko*, Hemidactylus turcicus. [Master thesis]. Southeastern Louisiana University, Hammond, Louisiana, USA.

Gribbins, K. (2011). Reptilian spermatogenesis: A histological and ultrastructural perspective. *Spermatogenesis*, *1*(3), 250–269. https://doi.org/10.4161/spmg.1.3.18092

Gribbins, K., & Rheubert, J. L. (2011). The ophidian testis, spermatogenesis, and mature spermatozoa. In R. D. Aldridge & D. M. Sever (Eds.), *Reproductive biology and phylogeny of snakes* (pp. 183–264). Science Publishers.

Gribbins, K., & Rheubert, J. L. (2014). The architecture of the testis, spermatogenesis, and mature spermatozoa. In J. L. Rheubert, D. S. Siegel, & S. E. Trauth (Eds.), *Reproductive biology and phylogeny of lizards and tuatara* (pp. 340–424). CRC Press.

Guedes, T. B., Entiauspe-Neto, O. M., & Costa, H. C. (2023). Lista de répteis do Brasil: atualização de 2022. *Herpetologia Brasileira*, *12*(1), 56–161.

Halliday, T. (1994). *Animal behavior*. University of Oklahoma Press.

Harvey, M. B., Ugueto, G. N., & Gutberlet Jr, R. L. (2012). Review of teiid morphology with a revised taxonomy and phylogeny of the Teiidae (Lepidosauria: Squamata). *Zootaxa*, *3459*(1), 1. https://doi.org/10.11646/zootaxa.3459.1.1

Hildebrand, M., Goslow, G. E., & Hildebrand, V. (2006). *Análise da estrutura dos vertebrados*. Atheneu Editora.

Howat, W. J., & Wilson, B. A. (2014). Tissue fixation and the effect of molecular fixatives on downstream staining procedures. *Methods*, *70*(1), 12–19. https://doi.org/10.1016/j.ymeth.2014.01.022

Kahrl, A. F., & Cox, R. M. (2015). Diet affects ejaculate traits in a lizard with condition-dependent fertilization success. *Behavioral Ecology*, *26*(6), 1502–1511. https://doi.org/10.1093/beheco/arv105

Knotek, Z., Cermakova, E., & Oliveri, M. (2017). Reproductive medicine in lizards. *Veterinary Clinics: Exotic Animal Practice*, *20*(2), 411–438. https://doi.org/10.1016/j.cvex.2016.11.006

Loebens, L., Cechin, S. Z., Theis, T. F., Moura, L. B., & Almeida-Santos, S. M. (2017). Reproductive biology of *Philodryas patagoniensis* (Snakes: Dipsadidae) in South Brazil: Male reproductive cycle. *Acta Zoologica*, *98*(4), 329–339. https://doi.org/10.1111/azo.12172

Lüpold, S., Linz, G. M., Rivers, J. W., Westneat, D. F., & Birkhead, T. R. (2009). Sperm competition selects beyond relative testes size in birds. *Evolution*, *63*(2), 391–402.

Mendes, R. M. M., Pinheiro, N. L., Nascimento, A. A., Santos, C. M., Ribeiro, T., Santos, M. A., & Sales, A. (2009). Histologia comparada de testículo e do segmento sexual do rim de lagarto tropical *Tropidurus torquatus*. *Revista de Ciência da Vida*, *29*(1), 49–54.

Migliore, S. N., Ramalho, R. A., Araújo, G. S., & Almeida-Santos, S. M. (2024). Cycle of the sexual segment of the kidney: Histological insights into the role of the urinary tract in the reproduction of male *Notomabuya frenata* (Squamata: Scincidae). *Zoology*, *162*, 126146. https://doi.org/10.1016/j.zool.2024.126146

Novelli, I. A., Oliveira, P. R. D. E., Castanon, M. C. M. N., Silva, P. C., & Sousa, B. M. (2018). Morphological and histological characterization of sexual segment of the kidney in *Notomabuya frenata* (Cope, 1862) and *Aspronema dorsivittatum* (Cope, 1862) (Squamata, Mabuyidae). *Anais da Academia Brasileira de Ciências*, *90*(2 suppl 1), 2267–2278. https://doi.org/10.1590/0001-3765201820170871

Nunes, P. M. S. (2011). *Morfologia hemipeniana dos lagartos microteídeos e suas implicações nas relações filogenéticas da família Gymnophthalmidae (Teiioidea: Squamata)* [PhD Thesis]. Universidade de São Paulo.

Nunes, P. M. S., Fouquet, A., Curcio, F. F., Kok, P. J., & Rodrigues, M. T. (2012). Cryptic species in *Iphisa elegans* Gray, 1851 (Squamata: Gymnophthalmidae) revealed by hemipenial morphology and molecular data. *Zoological Journal of the Linnean Society*, *166*(2), 361–376. https://doi.org/10.1111/j.1096-3642.2012.00846.x

Pewhom, A., & Srakaew, N. (2018). Microanatomy of the testes and testicular ducts of the butterfly lizard, *Leiolepis ocellata* Peters, 1971 (Reptilia: Squamata: Agamidae) during the active reproductive period. *Acta Zoologica*, *101*(1), 51–68. https://doi.org/10.1111/azo.12270

R Development Core Team. (2016). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.Rproject.org/

Rheubert, J., Pasternak, M. A., Ely, M., Siegel, D. S., Trauth, S. E., Gribbins, K. M., & Sever, D. M. (2020). Seasonal histology and ultrastructure of the urogenital system in two sympatric lizards. *Journal of Zoology*, *310*(4), 273–286. https://doi.org/10.1111/jzo.12748

Rheubert, J. L., Sever, D. M., Geheber, A. D., & Siegel, D. S. (2010). Proximal testicular ducts of the Mediterranean Gecko (*Hemidactylus turcicus*). *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology*, *293*(12), 2176–2192. https://doi.org/10.1002/ar.21282

Rheubert, J. L., Sever, D. M., Siegel, D. S., & Trauth, S. E. (2014). Male reproductive anatomy: The gonadoducts, sexual segment of the kidney, and cloaca. In J. L. Rheubert, D. S. Siegel, & S. E. Trauth (Eds.), *Reproductive biology and phylogeny of lizards and tuatara* (pp. 253–301). CRC Press.

Rocha, C. F. D. (2008). Body size, female reproduction and sexual dimorphism in the lizard *Ameiva ameiva* (Teiidae) in a restinga of southeastern Brazil. *Revista Brasileira de Zoologia*, *25*, 370–372.

Rojas, C. A., Barros, V. A., & Almeida-Santos, S. M. (2013). The reproductive cycle of the male sleep snake *Sibynomorphus mikanii* (Schlegel, 1837) from southeastern Brazil. *Journal of Morphology*, *274*(2), 215–228. https://doi.org/10.1002/jmor.20099

Sever, D. M. (2010). Ultrastructure of the reproductive system of the black swamp snake (*Seminatrix pygaea*). VI. Anterior testicular ducts and their nomenclature. *Journal of Morphology*, *271*(1), 104–115. https://doi.org/10.1002/jmor.10784

Sever, D. M., & Hopkins, W. A. (2005). Renal sexual segment of the ground skink, *Scincella laterale* (Reptilia, Squamata, Scincidae). *Journal of Morphology*, *266*(1), 46–59. https://doi.org/10.1002/jmor.10364

Sever, D. M., Rheubert, J. L., Hill, T. A., & Siegel, D. S. (2013). Observations on variation in the ultrastructure of the proximal testicular ducts of the ground skink, *Scincella lateralis* (Reptilia: Squamata). *Journal of Morphology*, *274*(4), 429–446. https://doi.org/10.1002/jmor.20104

Sharath, J., Samson, S. K., & Bhagya, M. (2022). The efferent ductules in the lizard *Eutropis carinata*: A functional morphology and ultrastructural study. *South American Journal of Herpetology*, *23*(1), 49–57. https://doi.org/10.2994/SAJH-D-20-00023.1

Siegel, D. S., Sever, D. M., Rheubert, J. L., & Gribbins, K. M. (2009). Reproductive biology of *Agkistrodon piscivorus* Lacépède (Squamata, Serpentes, Viperidae, Crotalinae). *Herpetological Monographs*, *23*(1), 74–107. https://doi.org/10.1655/08-031.1

Siegel, D. S., Trauth, S. E., Sever, D. M., & Aldridge, R. D. (2011). The phylogenetic distribution of the ampulla ureter and ampulla urogenital/uriniferous papilla in the Serpentes. *Journal of Zoological Systematics and Evolutionary Research*, *49*(2), 160–168. https://doi.org/10.1111/j.1439-0469.2010.00576.x

Silva, M. B., de Lima-Filho, G. R., Cronemberger, Á. A., Carvalho, L. S., Manzani, P. R., & Vieira, J. B. (2013).

Description of the hemipenial morphology of *Tupinambis quadrilineatus* Manzani and Abe, 1997 (Squamata, Teiidae) and new records from Piauí, Brazil. *ZooKeys*, *361*, 61–72. https://doi.org/10.3897/zookeys.361.5738

Trauth, S. E. (2018). Distal urogenital anatomy of male southern coal skinks, *Plestiodon anthracinus pluvialis* (Reptilia: Scincidae). *Journal of the Arkansas Academy of Science*, *72*(1), 7–13.

Trauth, S. E. (2020). Distal urogenital anatomy of male prairie racerunners, *Aspidoscelis sexlineatus viridis* (Reptilia: Sauria: Teiidae). *Journal of the Arkansas Academy of Science*, *74*(1), 11–20. https://doi.org/10.54119/jaas.2020.7406

Trauth, S. E., & Sever, D. M. (2011). Male urogenital ducts and cloacal anatomy. In R. D. Aldridge & D. M. Sever (Eds.), *Reproductive biology and phylogeny of snakes* (pp. 411–476). Science Publishers.

Villagrán-SantaCruz, M., Hernández-Aguacaliente, M. J., Méndez-de la Cruz, F. R., Gribbins, K. M., Granados-González, G., & Hernández-Gallegos, O. (2014). The germ cell development strategy and seasonal changes in spermatogenesis and Leydig cell morphologies of the spiny lizard *Sceloporus mucronatus* (Squamata: Phrynosomatidae). *Zoomorphology*, *133*, 151–165. https://doi.org/10.1007/s00435-013-0210-7

Vitt, L. J., & Colli, G. R. (1994). Geographical ecology of a neotropical lizard: Ameiva ameiva (Teiidae) in Brazil. *Canadian Journal of Zoology*, *72*(11), 1986–2008.

Vitt, L., Magnusson, W. E., Ávila-Pires, T. C., & Lima, A. P. (2008). *Guia de lagartos da Reserva Adolpho Ducke*. Áttema Design Editorial.

Yoshida, S. (2016). From cyst to tubule: Innovations in vertebrate spermatogenesis. *Wiley Interdisciplinary Reviews: Developmental Biology*, *5*(1), 119–131. https://doi.org/10.1002/wdev.204

Zar, J. (1999). *Biostatistical analyses*. Printice Hall.

Zieri, R., Taboga, S. R., & Oliveira, C. (2008). Espermiogênese em *Eupemphix nattereri* (Anura, Leiuperidae): aspectos ultra-estruturais. *Iheringia*, *98*(2), 193–199. https://doi.org/10.1590/s0073-47212008000200004

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