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Spermatozoa in the sexual segment of the kidney and urogenital morphology of male Monocled Cobras, *Naja kaouthia* Lesson, 1831, from Ang Thong Province, Thailand

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Abstract. The Monocled Cobra (*Naja kaouthia*) is a medically important elapid, widely distributed across South and Southeast Asia. However, the functional roles of the urogenital system, particularly the secretions of the sexual segment of the kidney (SSK) and their interactions with spermatozoa in male squamates remain poorly understood. We examined the macro- and microscopic anatomy of the male urogenital system in seven adult *N. kaouthia* specimens obtained post-mortem from local farmers in Ang Thong Province, central Thailand. Histological analyses revealed testes in recrudescence, spermiogenesis, and spermiation stages, with spermatozoa present in the ductus epididymis and ductus deferens during active testicular stages. The kidneys exhibited marked variation in SSK development, varying from hypotrophied to maximally hypertrophied tubules. Notably, spermatozoa were observed within the lumen of the SSK, collecting ducts, and ureters. These findings provide anatomical evidence that contributes to the understanding of the functional role of the SSK in elapid snakes and other squamates.

Keywords. Histology, Male reproductive tract, Renal system, Serpentes, Spermiogenesis

Introduction

Among reptiles, squamates possess particularly distinctive male reproductive and urinary systems, characterised by specialised anatomical and functional adaptations. Sperm produced in the seminiferous tubules of the testes is transported through a series of testicular ducts, including the rete testis, ductuli efferentes, ductus epididymis, and ductus deferens (Trauth and Sever, 2011). In addition to excretion, the kidney of male squamates contains a testosterone-dependent secondary sexual structure known as the sexual segment of the kidney (SSK) (Prasad and Reddy, 1972; Sever et al., 2002), formed by modifications of the distal tubules of the renal system (Prasad and Reddy, 1972). Numerous studies have documented seasonal changes in the morphology and activity of the SSK across diverse squamate taxa, particularly in snakes (Aldridge et al., 2011). The SSK has been proposed to serve several reproductive functions, including that its secretions are associated with sperm maintenance within the female oviduct (Aldridge et al., 2011; Friesen et al., 2013; Silva et al., 2022) and with copulatory plug formation (Sever et al., 2002; Friesen et al., 2013). However, the precise functional roles of SSK secretions and their interactions with spermatozoa remain incompletely understood (Aldridge et al., 2011; Migliore et al., 2024).

Here, we describe the macro- and microscopic anatomy of the urogenital system in adult male Monocled Cobra (*Naja kaouthia* Lesson, 1831) from Ang Thong Province, central Thailand, including the reproductive components of the testes and testicular ducts, and the urinary components of the kidneys and the SSK. We also report on the presence of spermatozoa within the SSK, collecting ducts, and ureters. These findings provide evidence of changes in both external and internal anatomy that may contribute to the functional role of the SSK in elapid snakes and other squamates.

Materials and Methods

Specimen collection. Seven adult male *Naja kaouthia* were obtained opportunistically from local farmers following accidental deaths in paddy fields of Ang

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Thong Province, Thailand (14.4906°N, 100.3181°E; 7 m elevation), during August–September 2020. Sex was determined based on external morphology, with males exhibiting thicker and longer tails than females, and was confirmed by macroscopic examination of the gonads. Adults were identified by a snout-vent length (SVL) ≥ 100 centimetres (cm) and body mass (BM) > 700 grams (g), following Chaitae (2011). All specimens were transported to the Physiology Laboratory, Department of Zoology, Faculty of Science, Kasetsart University, for morphometric measurements and dissection.

Macro– and microscopic study of reproductive organs. Specimens were measured for SVL, tail length (TL), and total body length (TBL), and were dissected ventrally to examine the internal morphology of the urogenital organs. The SVL, TL, and TBL were measured in centimetres (cm) using a measuring tape, and BM was recorded in grams (g) using a digital scale. Testicular length, width, and thickness were measured in millimetres (mm), and testicular weight was recorded in grams. Testicular volume (TV) was calculated following Pleguezuelos and Feriche (1999) using the equation: $TV = 4/3 \times \pi abc$, where a is half the length; b is half the width; and c is half the thickness of the testes. Kidney length and width were measured in mm, and kidney weight was recorded in g. Urogenital organs were preserved in 10% NBF for 48 hours. Organs from the right side were processed following standard histological procedures: tissues were dehydrated in a graded ethanol series (70%, 80%, 95%, and 100%), cleared in xylene, and embedded in paraffin. Sections of 5 µm thickness were then cut and stained with haematoxylin and eosin (H&E). Epithelial height was measured in µm from 20 epithelial cells per condition. The ductus epididymis was classified by the absence or presence of spermatozoa, and the ductus deferens by few or abundant spermatozoa in the lumen. SSK diameter was measured in µm from 10 transverse tubule sections per sample, as described by Loebens et al. (2017).

Statistical analysis. Data are expressed as mean ± standard deviation (SD). Normality was assessed using the Kolmogorov–Smirnov test (Massey, 1951). As the data of epithelial height were non-normally distributed, the Mann–Whitney U test (Mann and Whitney, 1947) was used for group comparisons, with statistical significance set at $p < 0.05$.

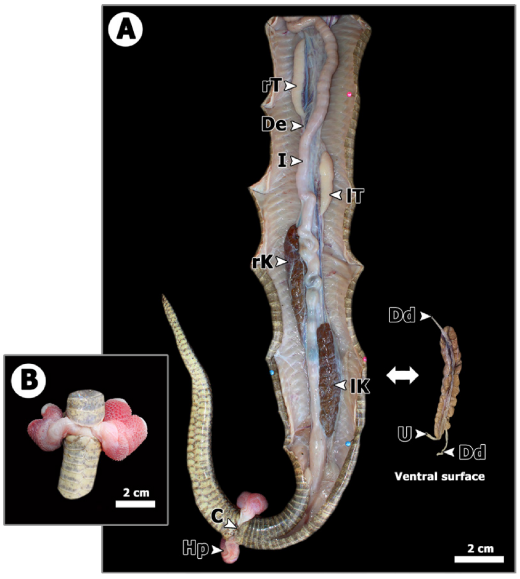

Figure 1. Macroscopic morphology of male urogenital organs of *Naja kaouthia*. (A) Male urogenital organs; (B) Hemipenes inflated with water. Abbreviations: C; Cloacal opening, Dd; Ductus deferens, De; Ductus epididymis, Hp; Hemipenis, I; Intestine, lK; Left kidney, lT; Left testis, rK; Right kidney, rT; Right testis, and U; Ureter.

Results

Macroscopic morphology of urogenital organs. The male urogenital organs of *Naja kaouthia* were located in the posterior third of the coelomic cavity and comprised right and left reproductive and excretory components. The right urogenital organs were positioned anterior to the left, and the testes were located above the kidneys. Each elongated testis was connected to a ductus epididymis, and subsequently a ductus deferens, which ran parallel to the ventral surface of the corresponding kidney along with the ureter (Fig. 1A). Eventually, both the ductus deferens and ureter extended into the cloacal opening. The ductus deferens opened into the bilobed hemipenes, which were densely covered with small spines (Fig. 1B) and were enclosed within the tail. We observed variations in the macroscopic morphometry of the testes and kidneys, as shown in Table 1.

Microscopic morphology of male reproductive organs. Based on histological examination of seven adult male *N. kaouthia*, spermatozoa were produced in the seminiferous tubules and transported through the system of testicular ducts as follows: rete testis, ductuli efferentes, ductus epididymis, and ductus deferens. Based on the limited sample size, complete regression of the testis was not observed in this study. Instead, we observed the recrudescence stage

Table 1. Morphometric measurements of the male urogenital organs of *Naja kaouthia*.

Traits	*n* = 7	
	Mean ± SD	Ranges
Body measurements		
Snout-vent length (cm)	125.24 ± 6.86	114–134
Tail length (cm)	22.41 ± 2.23	18–25
Total body length (cm)	147.66 ± 7.04	135.9–159
Body mass (g)	985.71 ± 348.47	700–1700
Testicular morphometry		
Right testicular weight (g)	1.78 ± 1.17	0.52–3.64
Left testicular weight (g)	1.53 ± 0.92	0.49–2.86
Average testicular weight (g)	1.65 ± 1.04	0.49–3.64
Right testicular volume (mm^3)	703.86 ± 429.28	257.54–1421.27
Left testicular volume (mm^3)	695.96 ± 515.48	176.51–1648.13
Average testicular volume (mm^3)	699.91 ± 464.52	176.51–1648.13
Kidney morphometry		
Right kidney weight (g)	5.16 ± 2.47	2.74–8.64
Left kidney weight (g)	4.94 ± 2.41	2.76–8.28
Average kidney weight (g)	5.05 ± 2.44	2.74–8.64
Right kidney length (mm)	68.90 ± 18.29	31.37–89.09
Left kidney length (mm)	67.89 ± 7.02	57.09–76.89
Average kidney length (mm)	62.20 ± 21.55	31.37–89.09
Right kidney width (mm)	13.26 ± 4.09	7.87–18.82
Left kidney width (mm)	13.72 ± 3.52	7.52–17.89
Average kidney width (mm)	12.42 ± 4.49	7.52–18.82

(testicular volume (TV) = 433.02 ± 194.53 mm^3, range = 217.03–594.43 mm^3, n = 3), spermiogenesis stage (TV = 688.53 ± 392.43 mm^3, range = 505.86–1139.00 mm^3, n = 3), and spermiation stage (TV = 1534.70 mm^3, n = 1). In these stages, the seminiferous epithelium exhibited a combination of Sertoli cells and germ cells at different stages of differentiation, whereas the interstitial tissue, containing blood vessels and Leydig cells, was distributed throughout the intertubular space. During testicular recrudescence, Sertoli cells and spermatogonia were located near the basement membrane of the seminiferous epithelium, followed by primary and secondary spermatocytes, as well as primary and secondary spermatids (Fig. 2A). Spermiogenesis was characterised by the accumulation of mature spermatozoa adjacent to the lumen of the seminiferous tubules (Fig. 2B). In the spermiation stage, spermatozoa were released into the lumen of the seminiferous tubules, completing the spermiation process (Fig. 2C, D). Across the studied specimens, the lumen of the ductus epididymis was empty (Fig. 2E), while only a few spermatozoa were observed in the lumen of the ductus deferens in males at the testicular recrudescence stage (Fig. 2F). Dense aggregations of spermatozoa were found in both the ductus epididymis (Fig. 2G) and ductus deferens (Fig. 2H) in males at the spermiogenesis and, particularly, the spermiation stages. The epithelial height of the ductus epididymis was significantly greater during the recrudescence stage, in which spermatozoa were absent, compared with those ducts containing spermatozoa (U = 97.50, p = 0.006). In contrast, the epithelial height of the ductus deferens did not differ between the testicular recrudescence stage and the active testicular stages (U = 184.00, p = 0.665) (Table 2).

Male sexual segment of the kidney (SSK) and sperm in the ureter. The kidneys of male *N. kaouthia* were brownish-red, elongated, and exhibited morphological variation. Noticeable differences in external morphology were observed among individuals. In five specimens, including those in the testicular recrudescence and spermiogenesis stages, the kidneys displayed prominent vascular structures, with tubule-like structures visible only in some areas (weight = 3.71 ± 1.78 g, range = 2.94–5.33 g, length = 64.77 ± 13.48 mm, range = 31.37–80.62 mm, width = 11.96 ± 3.14 mm, range = 7.52–16.05 mm, n = 5) (Fig. 3A). In contrast, in two males at the spermiogenesis and spermiation stages, distinct tubule-like structures were evident throughout the kidney (weight = 8.39 ± 0.10 g, range = 8.19–8.64 g, length = 77.44 ± 13.48 mm, range = 69.29–89.09 mm, width = 17.32 ± 1.26 mm, range = 16.27–18.82 mm, n = 2) (Fig. 3B). Histological analysis confirmed that the tubule-like structures corresponded to the SSK. As in other male squamates, the kidney consisted of distinct nephron components, including renal corpuscles, proximal and distal convoluted tubules, and collecting ducts. The SSK was characterised by simple columnar epithelial cells with nuclei located near the basal region,

Figure 2. Microscopic morphology of the male genital organs of the *Naja kaouthia*. **(**A) Testicular recrudescence; (B) Spermiogenesis; (C, D) Spermiation; (E) Empty lumen of the ductus epididymis; (F) Low density of spermatozoa in the lumen of ductus deferens; (G) Lumen of ductus epididymis filled with spermatozoa; (H) Lumen of ductus deferens filled with spermatozoa. Abbreviations: Bv; Blood vessel, Ct; Connective tissue, Dde; Ductus deferens epithelium, De; ductuli efferentes, Dee, Ductus epididymis epithelium, Ic; Interstitial cell, Lu; Lumen, Mu; Muscular layer, S; Sertoli cell, Sc I; Primary spermatocyte, Sc II; Secondary spermatocyte, Se; Seminiferous epithelium, Smg; Spermatogonium, Spz; Spermatozoa, St I; Primary spermatid, St II; Secondary spermatid.

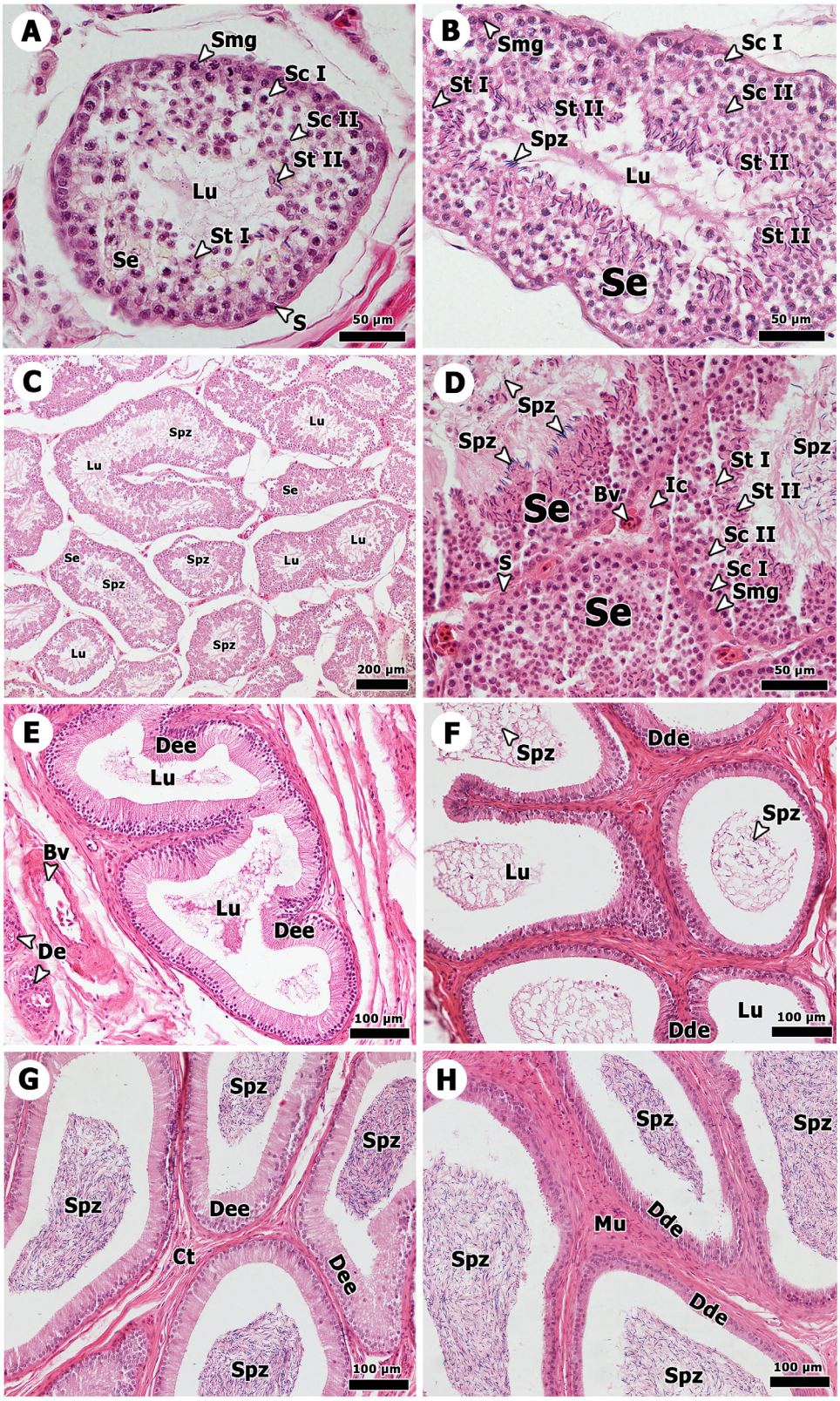
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Table 2. Epithelial height (μm) of the ductus epididymis and ductus deferens in adult male *Naja kaouthia*. Data are presented as mean ± SD. Statistical comparisons were performed using the Mann–Whitney U test. Asterisks indicate different levels of significance: * $p < 0.05$ and ** $p < 0.01$.

Structure	Epithelial height (μm)		Statistical test
	Absent or few spermatozoa in the lumen	Dense spermatozoa in the lumen	
Ductus epididymis	39.91 ± 4.58	35.65 ± 3.88	$U = 97.5$, $p = 0.006^{**}$
Ductus deferens	18.82 ± 2.72	19.47 ± 3.73	$U = 184.0$, $p = 0.665$

and tubules with a larger diameter compared to other renal tubules (SSK diameter = 83.70 ± 26.77 μm, range = 48.74–128.00 μm). Variation in SSK development was observed among individuals. Two males exhibited hypotrophied SSK (SSK diameter = 58.26 ± 11.43 μm, range = 41.83–81.06 μm) (Fig. 3C, D), while five males showed hypertrophied SSK with abundant eosinophilic secretory granules in the epithelial cells (SSK diameter = 93.88 ± 22.92 μm, range = 66.30–150.63 μm). In one male, the SSK reached maximal hypertrophy, with secretory granules present both within the epithelial cells and in the lumen of the tubules (SSK diameter = 128.00 ± 12.88 μm) (Fig. 3E, F). Additionally, spermatozoa were observed aggregated in the lumen of the ureters in two *N. kaouthia* (Fig. 4A, B). No abnormalities or ruptures of the urogenital organs were noted during dissection. Spermatozoa were also present within the lumen of the SSK and the collecting ducts (Fig. 4C, D). The transition between the SSK and the collecting duct was observed, with spermatozoa passing from the collecting duct into the SSK (Fig. 4E, F).

Discussion

In this study, the macro- and microscopic morphology of the male urogenital system was examined in seven adult *Naja kaouthia* obtained post-mortem from local farmers in Ang Thong Province, central Thailand. Based on external morphology, the overall arrangement of the male urogenital organs in *N. kaouthia* conforms to the typical pattern observed in most snakes (e.g., Halpert et al., 1982; Khouri et al., 2020; Thongboon et al., 2020), where the right urogenital organs are positioned more anteriorly than the left, as also reported in elapid snakes (Pewhom and Vanikasampanna, 2024). Each elongated testis was connected to a ductus epididymis and a ductus deferens, which ran parallel to the ventral surface of the kidney alongside the ureter. The ductus deferens opened into the bilobed, spinose hemipenes.

Three stages of testicular activity were identified based on histological observations, including recrudescence, spermiogenesis, and spermiation. A previous study by Chaitae (2011) suggested that the breeding season of *N. kaouthia* in the wild occurs from October to January, while captive populations breed between November and January (Chanhome et al., 2001). Tumkiratiwong et al. (2012) further demonstrated annual changes in testosterone levels and testicular stages in captive *N. kaouthia*, showing that testosterone levels gradually increased from January and reached a peak in October. These findings suggest that the specimens examined in the present study were collected during a period of elevated testosterone levels, while testicular activity was likely transitional between active spermatogenesis and the arrest phase.

We observed variation in macroscopic and microscopic morphology of the kidney and the development of the sexual segment of the kidney (SSK). Males in the testicular recrudescence and early spermiogenesis exhibited kidneys with prominent vascular patterns and poorly developed external tubulation, whereas males in late spermiogenesis and spermiation displayed conspicuous tubule-like structures as described by Aldridge et al. (2011). Seasonal variation in kidney macroscopic morphology has been reported in *Amerotyphlops brongersmianus* (Khouri et al., 2020); however, within the genus *Naja*, previous studies have been largely limited to microscopic variation, including *N. naja* (Saint Girons, 1972) and *N. samarensis* (Sever et al., 2012). A limited number of specimens revealed variation in SSK tubular diameter corresponding to different stages, including hypotrophied and hypertrophied stages. Similar patterns of SSK hypertrophy associated with reproductive activity have been reported in most snake species (Aldridge et al., 2011). In *N. kaouthia*, increased SSK hypertrophy coincided with the presence of spermatozoa in the ductus deferens, suggesting a functional association between SSK development and spermatozoa viability

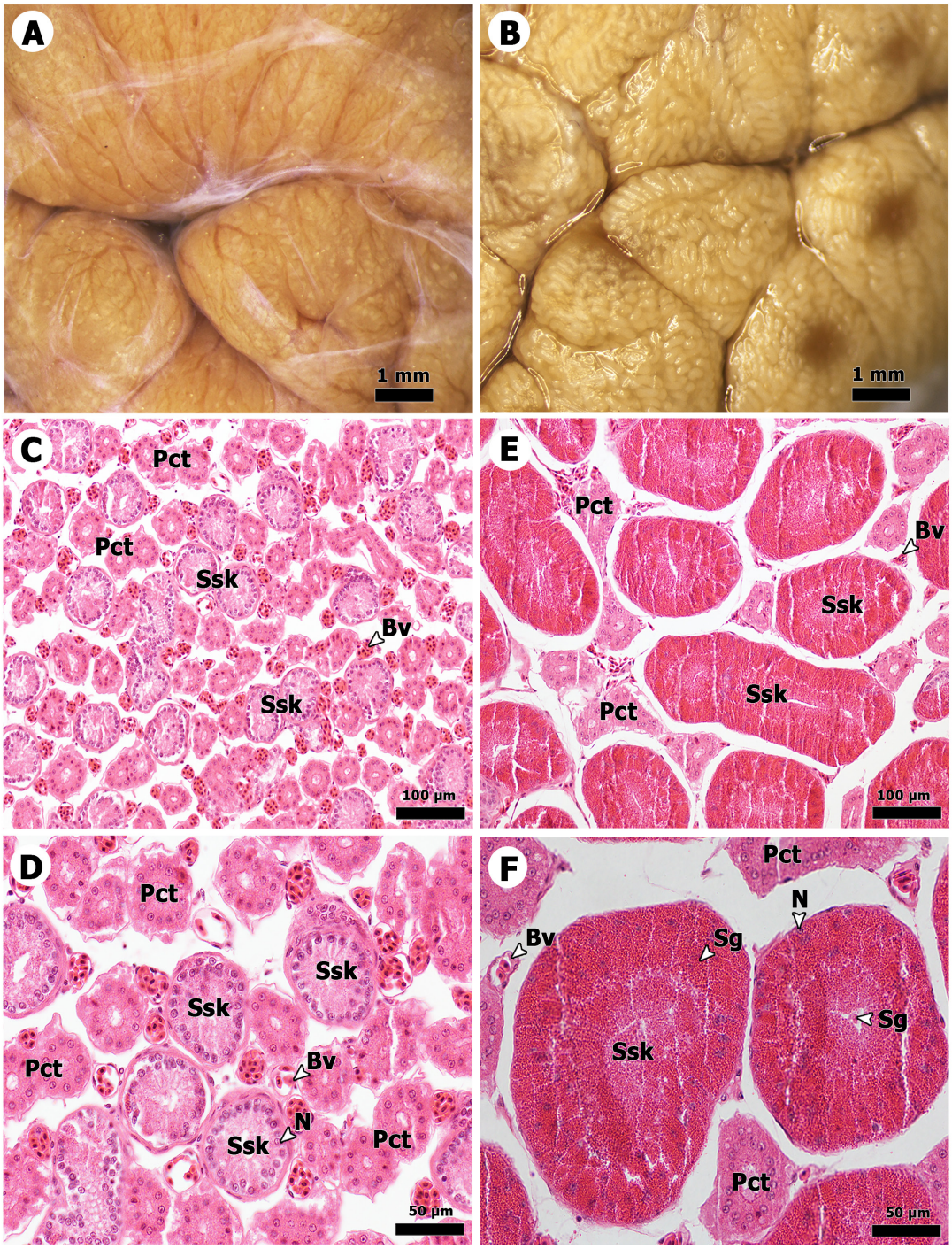

Figure 3. Macroscopic and microscopic morphology of the sexual segment of the kidney in male *Naja kaouthia*. (A, B). Macroscopic morphology of the male kidney showing variation among individuals. (C, D). Microscopic structure of hypotrophied Sexual segment of the kidney (SSK). (E, F). Microscopic structure of hypertrophied SSK with secretory granules in the epithelial cells and lumen. Abbreviations: Bv; Blood vessel, N; Nucleus, Pct; Proximal tubule, Sg; Secretory granule, Ssk; Sexual segment of the kidney.

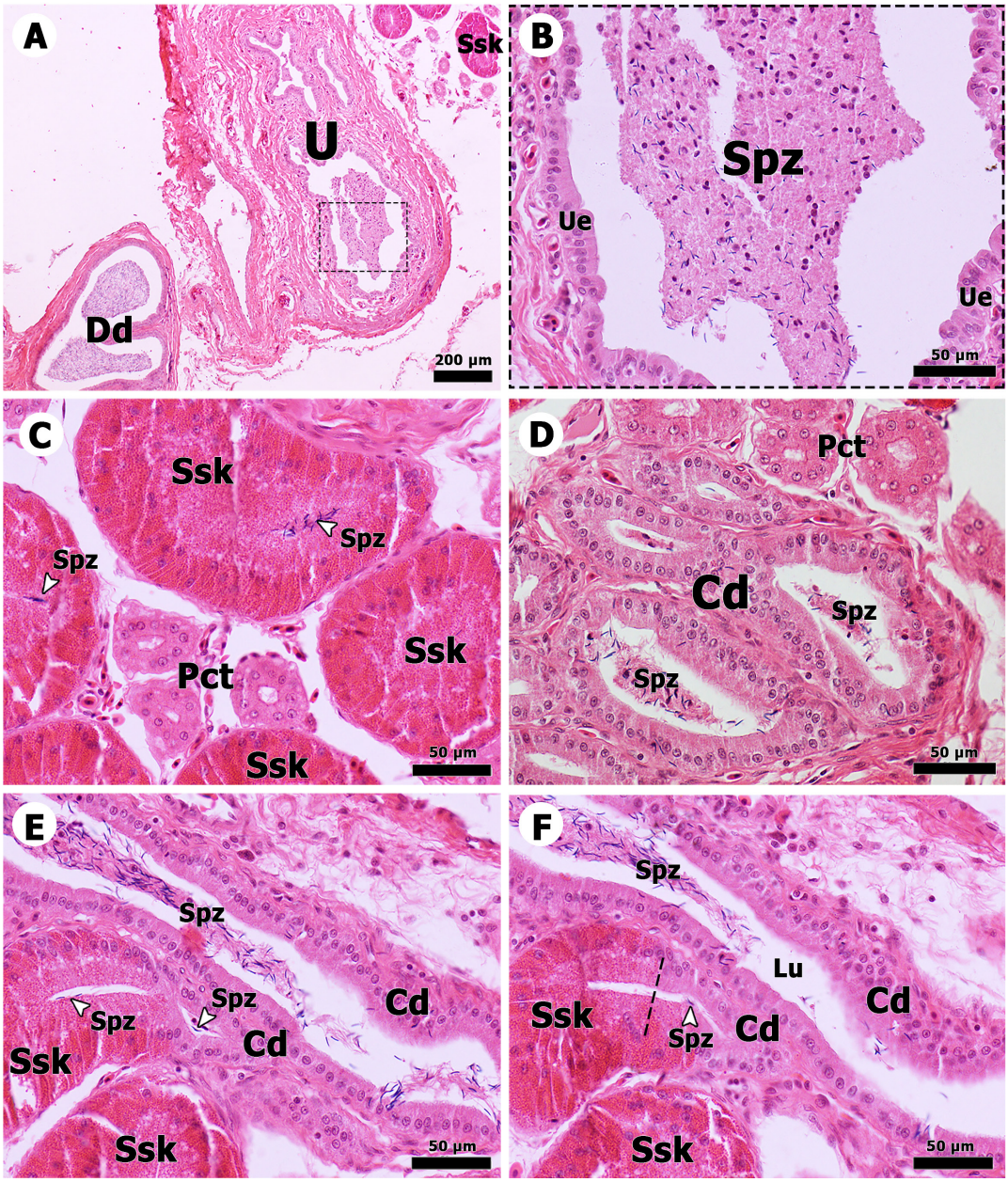

Figure 4. Sperm aggregation in the renal system of the male *Naja kaouthia*. (A, B) Aggregations of spermatozoa within the lumen of the ureters; (C) Spermatozoa observed in the lumen of the SSK; (D) Spermatozoa present within the collecting duct lumen; (E, F) Transition zone between the SSK and the collecting duct, showing spermatozoa passing from the collecting duct into the SSK. Abbreviations: Cd; Collecting duct, Dd; Ductus deferens, Lu; Lumen, Pct; Proximal tubule, Ssk; Sexual segment of the kidney, Spz; Spermatozoa, U; Ureter, Ue; Ureter epithelium.

in the ductus deferens (Sever et al., 2012; Silva et al., 2020).

Spermatozoa are typically transported through the testicular ducts of the male reproductive system (Trauth and Sever, 2011) and are not normally present within the

renal components of the urogenital system, including the SSK (Barros et al., 2017). In contrast, an unexpected finding of this study was the presence of spermatozoa within the ureters, collecting ducts, and SSK in two males. Reflux of spermatozoa into the renal system has previously been reported in snakes, including *Bothrops pubescens*, in which sperm were observed within the ureter and SSK (Barros et al., 2017), and in *A. brongersmianus*, where spermatozoa were detected within the SSK lumen (Khouri et al., 2020). The ampullar ureter merged with the ductus deferens and opened into the cloaca, which may result in reflux of spermatozoa into the urinary system (Trauth and Sever, 2011). Similar observations have also been reported in other squamate reptiles, such as skinks (*Notomabuya frenata)* (Migliore et al., 2024).

Here, we report spermatozoa within the lumen of the collecting duct in a snake. Migliore et al. (2024) demonstrated that collecting ducts produce both neutral and acid mucopolysaccharides throughout the year; taken together with our findings, this suggests that spermatozoa may be attracted to or maintained within the collecting duct by these secretions, supporting functional integration between the genital and urinary systems. However, the relationship between the genital and urinary systems remains poorly understood and requires further investigation, particularly with respect to physiological regulation and detailed anatomical characterisation. This study provides anatomical evidence that enhances understanding of urogenital system function in the Monocled Cobra (*Naja kaouthia*) and has implications for other elapid snakes and squamates.

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