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Morphology of the reproductive system in *Marmosa robinsoni* (Mammalia: Didelphidae)

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Marmosa robinsoni (Didelphidae) is a small-sized marsupial characterized by a prehensile tail, nocturnal, solitary, predominantly arboreal/semiscansorial habits, and the absence of a marsupium. Descriptions of the reproductive system remain scarce for most neotropical marsupials, despite this system representing a major distinguishing feature between metatherians and eutherians. This study describes the external morphology and histology of the male and female reproductive systems of *M. robinsoni*, as well as secondary sexual characters indicative of reproductive activity. Eight individuals (five females and three males) were collected, processed, and examined. The reproductive tracts were described, and morphological patterns previously reported for other marsupials were observed with respect to anatomical arrangement, histological composition, and characterization of the organs and ducts comprising this system. However, some species-specific features were identified when compared with the few other species for which these characters have been studied, including the number of bulbourethral glands, the non-bifid configuration of the glans penis, pigmentation of the tunica vaginalis in the testes, and the presence of a submammary pigmented patch. Examination of the correspondence between gametogenic status and secondary sexual characters indicated that mammary arrangement and the presence of prominent testes are reliable indicators of reproductive activity.

Keywords: Epithelium; gametogenesis; histology; marsupials.

Marmosa robinsoni (Didelphidae) es un marsupial de talla pequeña, caracterizado por tener cola prensil, ser nocturno, solitario y mayormente arbóreo/semiescansorial, y carecer de marsupio. Son muy escasas las descripciones del sistema reproductivo para la mayoría de los marsupiales del Nuevo Mundo, a pesar de tratarse de un factor distintivo entre metaterios y euterios. El presente trabajo describe la morfología externa y la histología de los sistemas reproductivos de machos y hembras de *M. robinsoni*, así como los caracteres sexuales secundarios indicadores de actividad reproductiva. Se colectaron, procesaron y estudiaron ocho individuos, cinco hembras y tres machos. Se describieron los tractos y se encontraron patrones morfológicos ya reportados para otros marsupiales en cuanto a disposición, composición histológica y caracterización de los órganos y ductos constituyentes de este sistema. Sin embargo, se encontraron algunas características propias de esta especie, cuando se compara con las pocas otras especies en donde estos caracteres se han estudiado, en aspectos como cantidad de glándulas bulbouretrales, conformación simple del pene, pigmentación de la tunica vaginalis en testículos y presencia de un parche pigmentado submamario. Al revisar la correspondencia entre el estado de gametogénesis y los caracteres sexuales secundarios se encontró que la disposición de las mamas y la presencia de testículos prominentes son indicadores honestos de actividad reproductiva.

Palabras clave: Epitelio, gametogénesis, histología, marsupiales.

Marsupials constitute a distinctive group within Mammalia. Their young are born at a highly altricial stage after a very short intrauterine gestation and must migrate to the maternal abdomen, where they attach to the mammary glands, either within or outside a marsupium, to complete development (Ceballos *et al.* 2002; Morales-Jiménez *et al.* 2004). The female reproductive system is likewise distinctive: the ureters enter the urinary bladder medially, separating the genital ducts and preventing fusion of the distal region into a single uterine body. Consequently, two uterine bodies are present, together with two lateral vaginae for sperm ascent and a medial vagina that functions as the birth canal, all open into a single urogenital sinus near the anus (Renfree and Shaw 2001).

The Robinson's mouse opossum, *Marmosa robinsoni,* is a member of the family Didelphidae, which comprises most American marsupials and currently includes 92 described species with broad distributions across South America (Voss and Jansa 2021). Individuals of this species are small (<200 g) and are distributed throughout the northern Neotropics, occurring discontinuously in Central America and on some islands of Belize, Honduras, and Panama, and in

South America from Trinidad and Tobago through northern Venezuela to west of the Andes in Colombia, extending southward to northern Peru (O'Connell 1983; Rossi *et al.* 2010; Pérez-Hernández 2016). The species occupies a wide variety of habitats, including dry and humid forests, secondary vegetation, and disturbed areas (Padilla-Rivera 2018), and occurs from sea level to approximately 1,400 m above sea level, with occasional records between 1,200 and 2,000 m (Gutiérrez *et al.* 2014; GBIF 2022). Individuals are primarily arboreal/semiscansorial, nocturnal, and mainly insectivorous, and exhibit a dark circumocular mask characteristic of the genus, cinnamon-brown dorsal pelage, yellowish-buff ventral pelage, a long prehensile tail exceeding head–body length, and absence of a marsupium (O'Connell 1983).

Marmosa robinsoni exhibits moderate sexual dimorphism, with males attaining greater body and cranial size and possessing proportionally larger upper canines (López-Fuster *et al.* 2000; Rossi *et al.* 2010); no evident sexual differences in pelage coloration have been reported. The species conforms to the general pattern described for small didelphids: females lack a marsupium, gestation is very short (≈13–15 days), and neonates are extremely altricial and attach directly to the nipples to complete postnatal development (O'Connell 1983; Voss 2013). Litters are relatively large (approximately 6–14 young), and development includes a prolonged period of lactation followed by dorsal transport after detachment from the nipples. Although field information remains limited, reproduction is thought to be seasonal or polyestrous, with early sexual maturity and a short life-history strategy consistent with small body size and high reproductive output (reviewed in O'Connell 1983).

Within the family Didelphidae, studies of reproductive morphology have been conducted for only a limited number of species, in females (e.g., Gonçalves *et al.* 2009; Cadena Bohórquez 2016; Sepúlveda-Vásquez *et al.* 2025) and in males (e.g., de Barros *et al.* 2013; Costa *et al.* 2015). Despite its broad distribution in the Neotropics, available information on *Marmosa robinsoni* remains minimal. Existing data are largely restricted to aspects of its ecology, distribution, taxonomy, phylogeography, and genetics (Reig 1968; O'Connell 1983; Gutiérrez *et al.* 2014; Padilla-Rivera 2018), whereas no studies have addressed the anatomy of its reproductive system, and even less is known about its microscopic morphology. Furthermore, the relationship between external morphological characters indicative of reproductive activity and the anatomy and histological organization of the reproductive tracts has not been investigated.

In the present study, we provide a macroscopic and microscopic description of the male and female reproductive tracts of *Marmosa robinsoni* and compare our findings with available descriptions for other didelphid marsupials. In addition, through documentation of external morphological characters associated with reproductive activity and sexual dimorphism, we provide baseline information for future analyses of the reproductive biology of this species.

Materials and methods

Field phase. The collection area corresponds to a fragment of tropical dry forest of approximately 6–8 ha located in the northeastern sector of the municipality of Aguachica, department of Cesar, Colombia, locally known as "El Bosque del Agüil." This isolated patch is embedded within an urban matrix and is situated at 8°18'56.9" N, 73°37'40.1" W, between 150 and 200 m above sea level. Vegetation is typical of the lower elevations of the Cordillera Oriental of the Colombian Andes, with a predominance of the families Caesalpinaceae, Fabaceae, Mimosaceae, Bignoniaceae, and Acanthaceae, among others. The area receives between 1,000 and 1,400 mm of annual precipitation and contains watercourses that support these plant formations, which harbor a considerable number of species of high ecological value (Rangel *et al.* 2012). *Marmosa robinsoni* had not previously been reported from this locality; therefore, this study constitutes a new record for the site.

Specimens were captured using Sherman live traps (23 × 9 × 7.5 cm) arranged along two to three transects approximately 40 m in length and separated from one another by ~20 m. Along each transect, six to nine traps were placed on the ground at intervals of 3–5 m in areas of dense vegetation. Two additional traps were positioned in the arboreal stratum at heights between 1.5 and 2 m. Traps were checked daily during five consecutive sampling days in September, November, and December 2022 and January 2023, in the morning hours, and bait was replaced in the afternoon following MINAM (2015). To maximize capture success and evaluate bait effectiveness, five different bait formulations were used (Table SD1).

For each captured individual, sex, presence of mammae and scrotum, body mass (g), and standard mammalian biometric measurements (Hall 1962)—total length, tail length, hind foot length, and ear length—were recorded, together with information on locality conditions (climate, vegetation, elevation), date, trap number, and apparent reproductive condition. Each specimen was photographically documented.

Female reproductive condition was assessed based on number and position of mammae, degree of mammary development, and abdominal distension following features described by Rueda *et al.* (2013); no fetuses attached to the nipples were observed. In males, testicular position and coloration (dark bluish in adults) and the presence of sexually dimorphic external characters were evaluated (Cuartas-Calle and Muñoz 2003). For both sexes, adult status was initially established based on large body size, using measurements reported by O'Connell (1983) for *Marmosa robinsoni chapmani* as reference.

Laboratory phase. Some captured animals were collected and transported to the laboratory, where anesthesia was induced with diethyl ether in a fume hood

prior to euthanasia by intracardiac injection of lidocaine (Roxicaína®) using a fine-gauge needle. Death was confirmed by the absence of reflex responses, respiratory movements, and cardiac activity (AVMA 2020).

Collected individuals were deposited in the Mammal Collection of the Natural History Museum of the Universidad Industrial de Santander (UIS), comprising three adult males (UIS-MHN-M-2199, UIS-MHN-M-2201, UIS-MHN-M-2208), four adult females (UIS-MHN-M-2170, UIS-MHN-M-2171, UIS-MHN-M-2172, UIS-MHN-M-2200), and one juvenile female (UIS-MHN-M-2207) (Table SD2). Additional individuals captured were photographed, measured, and assessed for sex and apparent reproductive condition based on external characters, and then released at the capture site; these captures included other mammal species (Figure SD3), all following biosafety recommendations of Mills *et al.* (1998).

Collected specimens were processed and prepared as scientific reference material (skins, skulls, skeletons, and/or fluid-preserved carcasses) following the guidelines for the use of wild mammals in research and education proposed by Sikes *et al.* (2019). Taxonomic identification followed Patiño (2022) and Voss and Jansa (2009).

Bodies separated from skins were fixed by injection with 10% buffered formalin and immersed in the same fixative for 24 hr. Specimens were then rinsed thoroughly in running water and transferred through a graded series of commercial ethanol (30–70%), in which they were stored and labeled following Romero-Almaraz *et al.* (2007).

Bodies were dissected through a ventral incision to expose the abdominal cavity and associated reproductive tracts. Tracts were photographed *in situ* to document their position and then removed and placed in Petri dishes for anatomical description. The following components were separated for detailed examination: in females, ovaries, oviducts, uterus, cervix, and vaginae; and in males, prostate, deferent ducts, bulbourethral glands, penis, and testes with epididymides and efferent ducts. Reproductive condition was subsequently confirmed through histological analyses.

Each organ and selected tract segments were fixed in Bouin's solution, dehydrated through a graded ethanol series, cleared in xylene, transitioned through xylene–paraffin, and embedded in paraffin (Surgipath Paraplastâ; Leica Biosystems). Histological sections 5–10 µm thick were obtained using a rotary microtome (Sakura Accu-Cut SRM©), mounted on slides, and stained with hematoxylin and eosin (Luna 1968). Sections were examined and photographed using a light microscope (Nikon Eclipse 55i©). Images were edited and labeled in Adobe Photoshop, and ImageJ software (Schneider *et al.* 2012) was used to generate scale bars.

In male gonads, the seminiferous epithelial cycle was evaluated using the tubular morphology method; stages were identified based on the overall composition of the seminiferous epithelium, including the presence of spermatogonia, spermatocytes, spermatids, and spermatozoa (de Barros *et al.* 2013; Khamas *et al.* 2014; Costa *et al.* 2015). In females, stages of ovarian folliculogenesis were characterized by assessing follicular development and classifying follicles as primordial, primary, secondary, or tertiary according to features such as granulosa cell layer thickness, differentiation of the follicular theca, antral size, and presence or absence of Graafian follicles, *corpora lutea* and *corpora albicantia* (Cadena Bohórquez 2016).

In addition, ducts of both sexes were described with respect to overall shape and tissue composition (Gonçalves *et al.* 2009; de Barros *et al.* 2013; Lima *et al.* 2013; Costa *et al.* 2015; Schimming *et al.* 2018). Finally, gonadal condition was related to the external morphological characters recorded for each collected individual.

Ethical statement. We collected the animals under the 'Permiso marco de recolección de especímenes de

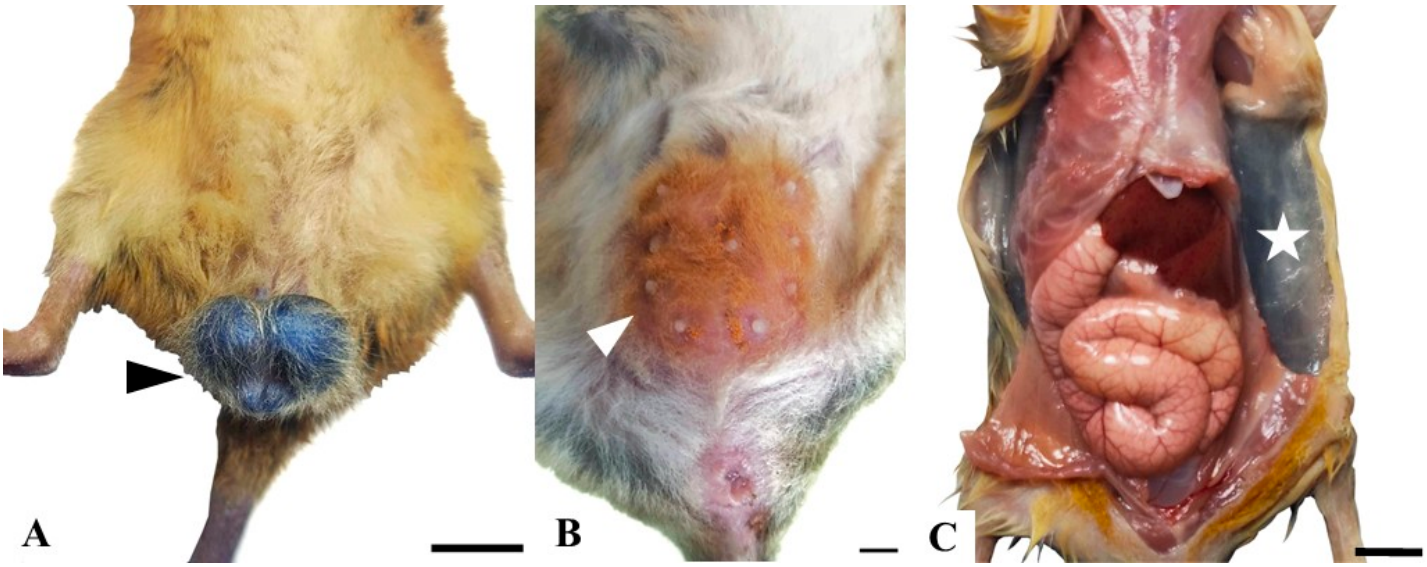

Figure 1. Secondary characteristics of sexual activity in *Marmosa robinsoni*, (A). Scrotal testes in adult reproductive males, black arrow. (B) nine abdominal breasts arranged in a circle, white arrow. (C) Submammary pigmented patch in the internal wall of the abdominal cavity, white asterisk. Scale bar, 10 mm.

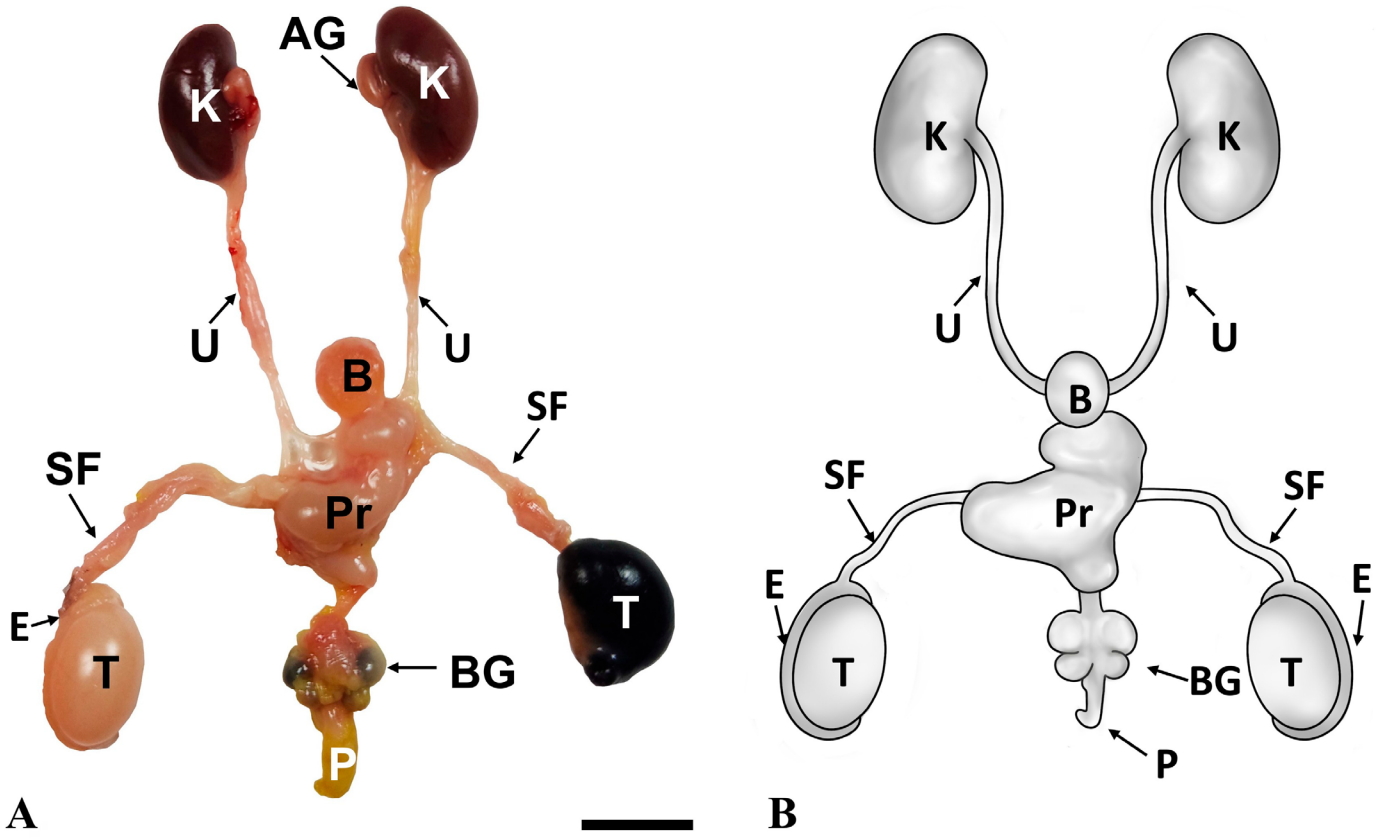

Figure 2. Male urogenital system of *Marmosa robinsoni*. (A) dissection; (B) graphical representation. AG, adrenal glands; B, bladder; BG, bulbourethral glands; E, Epididymis; K, kidneys; P, Penis; Pr, Prostate; SF, Spermatic funiculus; T, Testis; U, ureters. In the photograph, the testicle on the left is presented without the *tunica vaginalis*, so it allows us to observe the epididymis attached to the testis, while the right testis has the pigments of this layer giving it an intense dark color. Scale bar: 10 mm.

especies silvestres de la diversidad biológica con fines de investigación científica no comercial' (Autoridad Nacional de Licencias Ambientales ANLA, Resolution 0047, January 22nd, 2015). This study complies with all current Colombian laws and regulations concerning work with wildlife and adheres to ASM guidelines (Sikes *et al.* 2019).

Results

Eight individuals were captured, four adult females, one juvenile female, and three adult males, in addition to other individuals of this and other small mammal species (Figure SD3). Mean adult total length was 288.5 mm (SD = 5.12) in females and 267.3 mm (SD = 11.26) in males (Table SD2, Figure SD4). In males, testes were conspicuous and readily visible externally, with a mean maximum diameter of 6.5 mm (SD = 0.35) and intense dark bluish coloration in scrotal testes (Figure 1A). In females, mammary development was evident, with seven to 11 prominent nipples arranged in a circular pattern on the abdomen; additionally, pelage in this region exhibited a brownish-orange or rust coloration (Figure 1B). Along with this evident mammary development, a pigmented submammary patch was observed on the inner wall of the females' abdominal cavity (Figure 1C). Despite the prominence of mammary tissue in adult females, none appeared to be actively lactating.

Gross morphology of reproductive tracts. Exposure of the abdominal cavity (Figure SD5) allowed removal and examination of the reproductive systems of males and females. For improved visualization, the liver (Figure SD6B) and digestive tract (Figure SD6A) were displaced, and individual components of each reproductive tract were identified.

The male urogenital system measures approximately 5.5 cm in total length (measured in extension from the kidneys -*renes*- to the apex of the penis -*penis*-), is located in the lower abdominal cavity, and is composed of kidneys, ureters (*ureteres*), urinary bladder (*vesica urinaria*), spermatic cord (*funiculus spermaticus*), testes (*testes*), prostate (*prostata*), bulbourethral glands (*glandulae bulbourethrales*), and penis (Figure 2).

The penis is positioned within the inguinal cavity on the ventral plane, located caudal to the scrotal testes, and represents the distal termination of the urethra (*urethra)*; it is simple and non-bifid (Figure 3A). Testes are large, inguinal, darkly pigmented, and oval. Beneath the pigmented *tunica vaginalis* surrounding the testes, a whitish *tunica albuginea* and a laterally attached epididymis (*epididymis*) are evident. The epididymis is divided into three regions: head, body, and tail (*caput epididymidis*, *corpus epididymidis*, and *cauda epididymidis*) (Figure 3B). From the tail, the deferent duct (*ductus deferens*) enters the abdominal cavity within the spermatic cord, courses lateral to the ureters and terminates in the dorsal wall of the prostatic urethra. The prostate is composed of three segments closely associated with the urethra: a small proximal segment contiguous with the urinary bladder, a second and largest curved segment, and

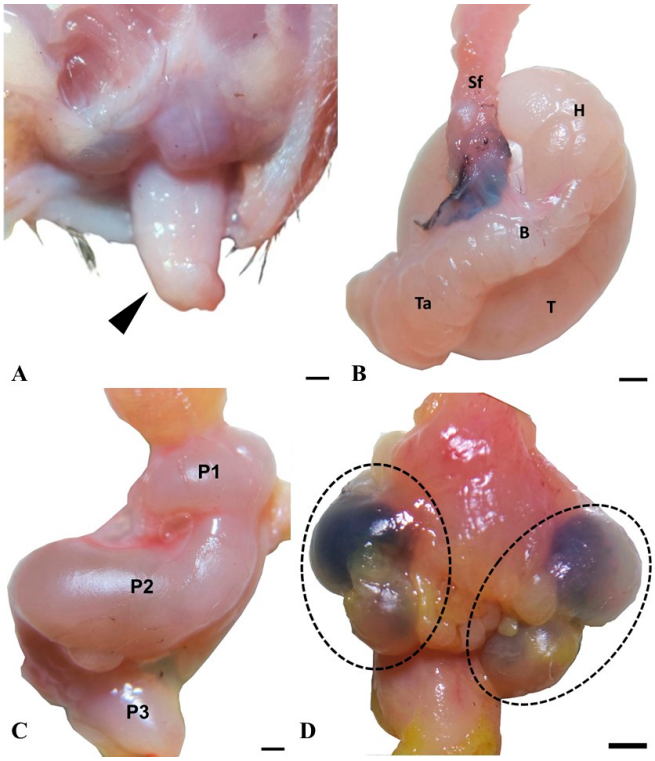

Figure 3. Male reproductive organs in *M. robinsoni*. (A) Non-bifid penis; (B) Testis; (C) Prostate; (D) Bulbourethral glands. Arrow, non-bifid penis; B, epididymal body; dotted line, left and right pair of bulbourethral glands; H, head of the epididymis; P1, prostate segment 1; P2, prostate segment 2; P3 prostate segment 3; Sf, spermatic funiculum; T, testis; Ta, epididymal tail. Scale bar, 1 mm.

a third narrow segment that extends to the membranous urethra (Figure 3C). Bulbourethral glands in *M. robinsoni* are in the perineal region and open near the origin of the penile urethra. They are rounded in shape and arranged as two pairs on each side; the dorsal glands are larger than the ventral ones (Figure 3D).

Dissection of female *Marmosa robinsoni* revealed an internally conspicuous, thickened, oval patch of darkly pigmented tissue lining the deep dermis of the body wall. This pigmented patch is restricted to the submammary region and was not observed elsewhere in the body wall, in the juvenile female or in males (Figure 1C, SD5).

The female urogenital system measures approximately 5.5 cm from the ovaries (*ovaria*) to the most distal region, is located in the lower abdominal cavity, and is composed of kidneys, ovaries, uteri (*uteri*), vaginae (*vaginae*), and urinary bladder. Ovaries are paired, ovoid, and whitish, measuring approximately 3 mm in length and 2 mm in width, and are situated distal to the kidneys and cranial to the uteri. The uteri are paired, parallel, and brownish (darker than the ovaries), each with its own cervix (*cervix uteri*) opening into a vaginal sinus (*atrium vaginae*) that is subdivided into two lateral vaginae (*vaginae laterales*) and a medial vagina (*vagina mediana*) that serves as the birth canal. Finally, the vaginae open into a common urogenital sinus (*sinus urogenitalis*) (Figure 4).

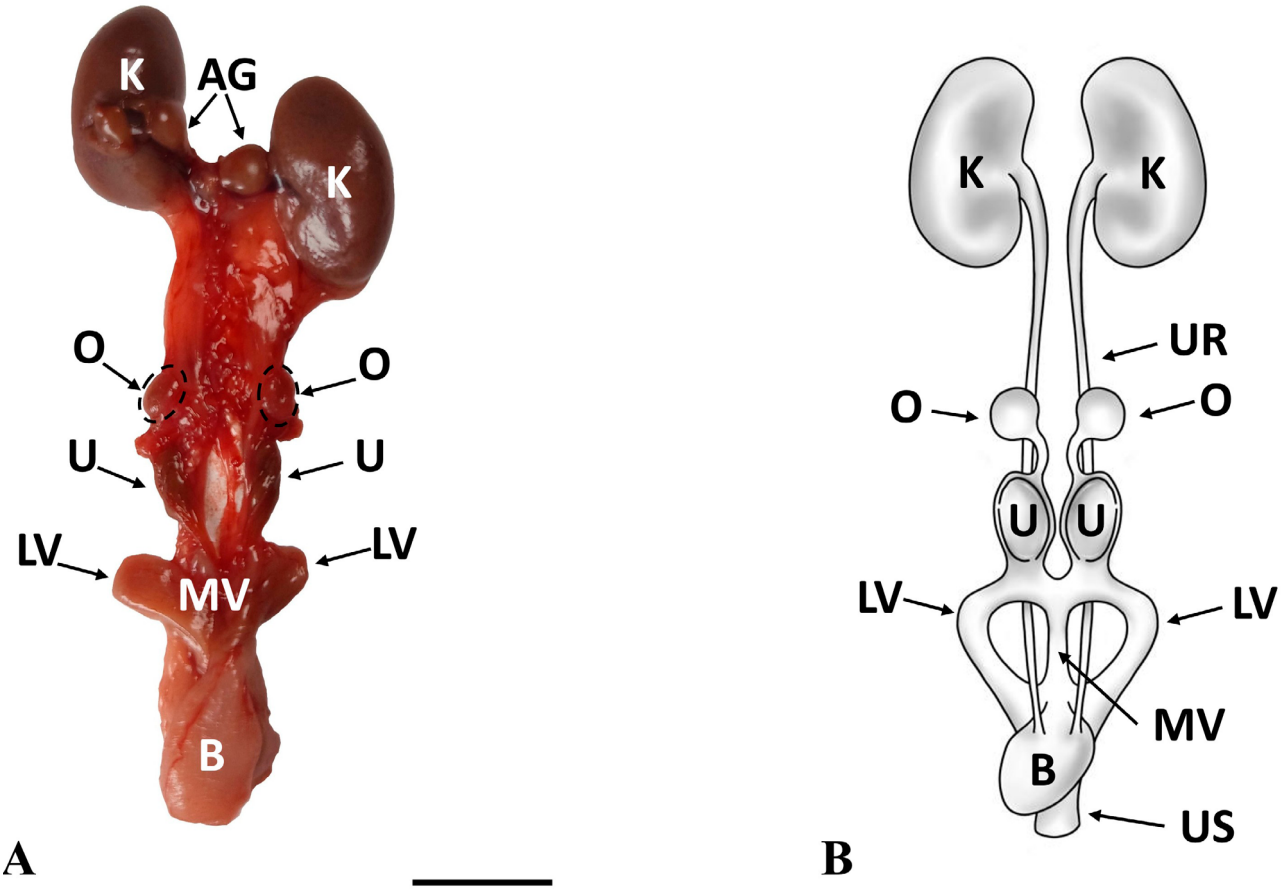

Figure 4. Female urogenital system of *Marmosa robinsoni*. (A) dissection; (B) graphical representation. AG, adrenal glands; B, Bladder; K, Kidneys; LV, Lateral vaginas; MV, Medial vagina; O, Ovaries; U, Uteri; UR, Ureters; US, Urogenital sinus. Scale bar10 mm.

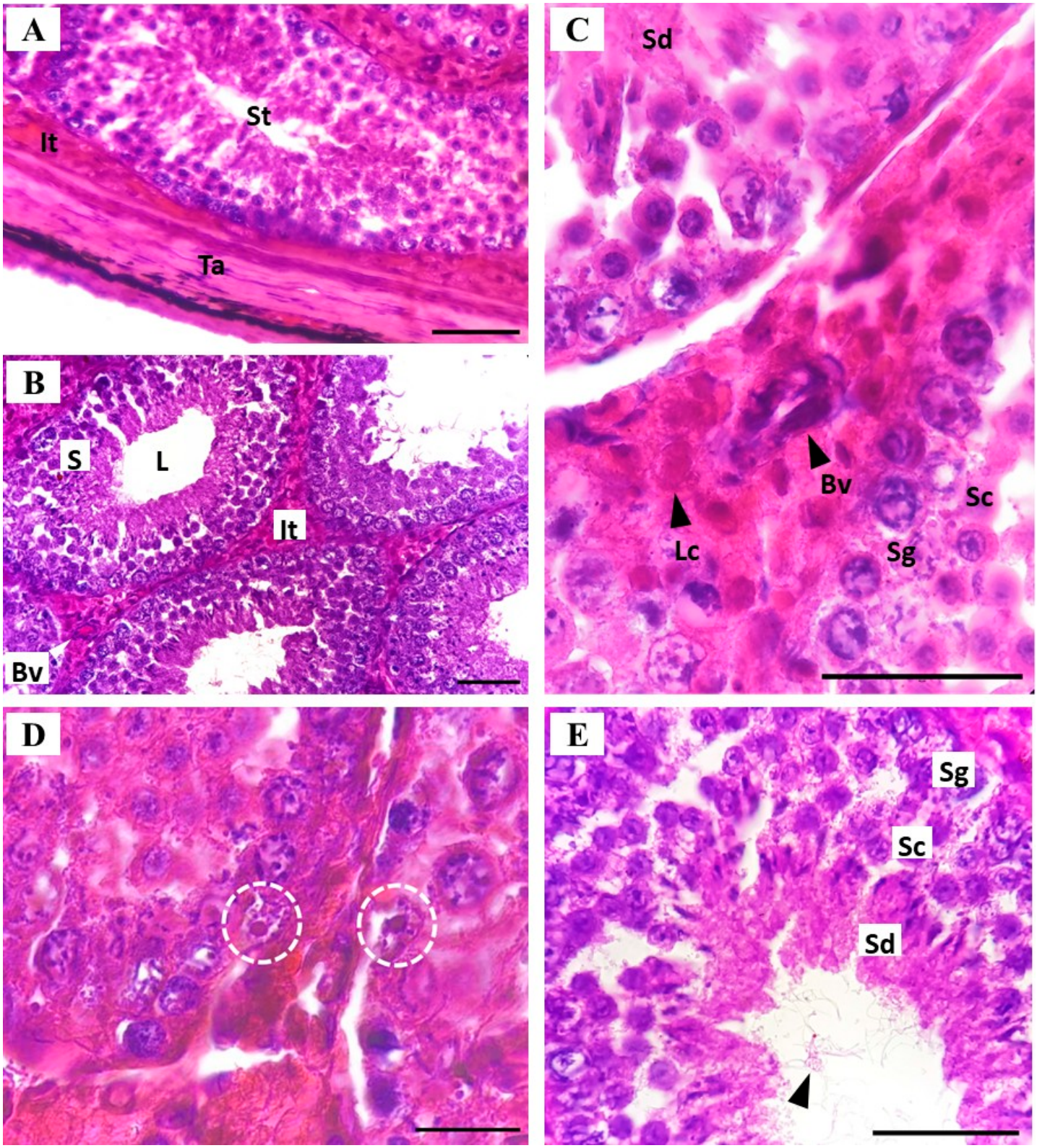

Figure 5. Histology of the testes of *Marmosa robinsoni*. (A) Testicular wall showing the *tunica albuginea,* the pigmentation in the *tunica vaginalis* and a seminiferous tubule. (B) Seminiferous tubules. (C) Detail of the interstitial tissue. (D) Detail of the basal region of a seminiferous tubule showing Sertoli cells enclosed in a white circle. (E) Detail of spermatic epithelium. Arrow, Sperm; Bv, Blood vessel; Dotted oval, Sertoli cells; It, Interstitial tissue; L, Lumen; Lc, Leidyg cells; S, Spermatic epithelium; Sc, Spermatocytes; Sd, Spermatids; Sg, Spermatogonia; St, Seminiferous tubule; Ta, *tunica albuginea*. Scale bar 50 *µm.*

Microscopic description male tract.

Testes. The testes are composed of seminiferous tubules (*tubuli seminiferi*) embedded within interstitial tissue and enclosed by the *tunica albuginea*, a layer of dense regular connective tissue (Figure 5A). Seminiferous tubules contain the germinal epithelium in which spermatogenesis proceeds in a centripetal direction, with spermatozoa located toward the tubular lumen (Figure 5B). Interstitial tissue consists of loose connective tissue containing Leydig cells, blood capillaries, vessels, and

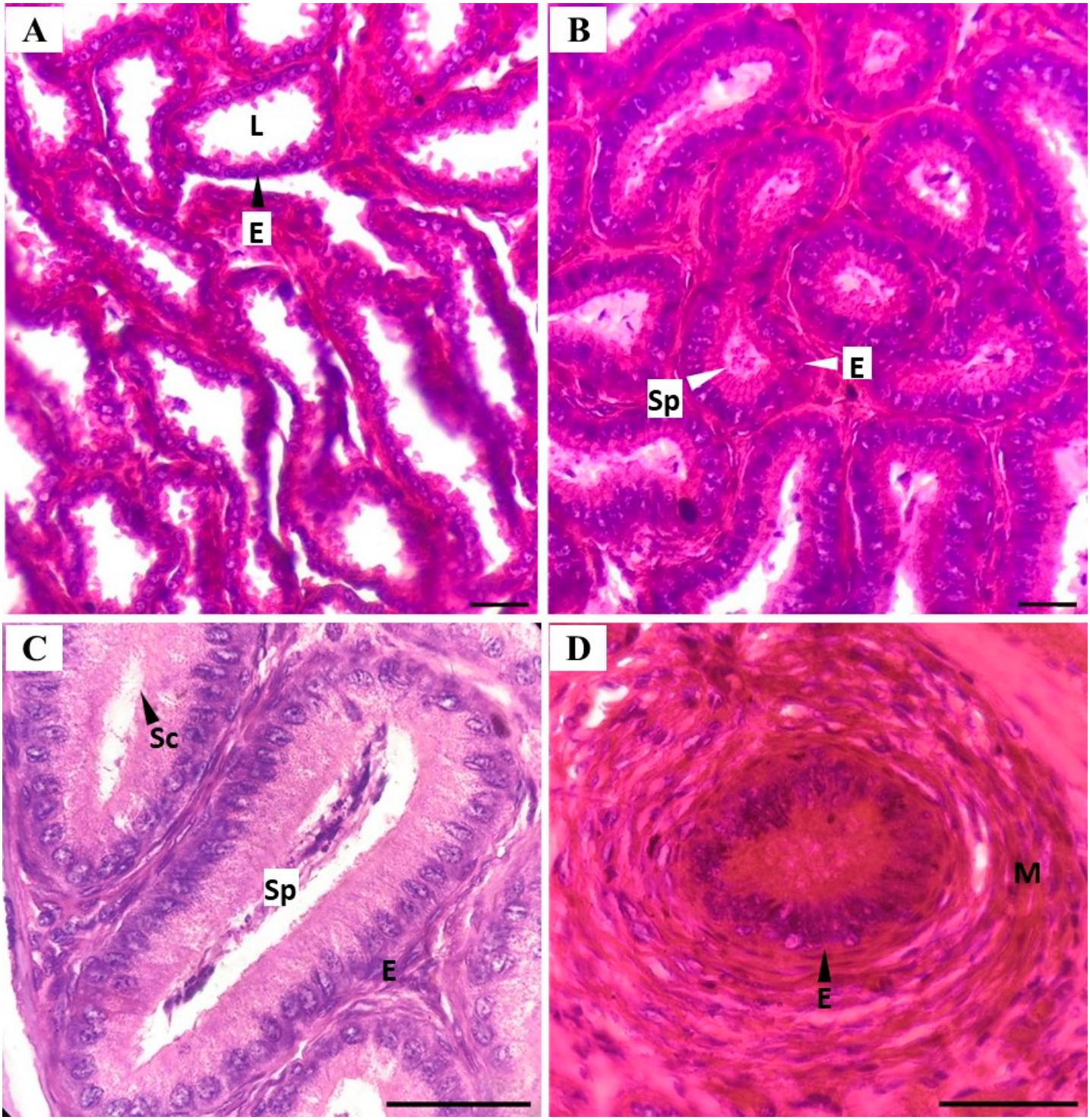

Figure 6. Histology of the excurrent ducts of *Marmosa robinsoni* male. (A) *Rete testis*; (B) Efferent ducts; (C) Epididymal ducts; (D) Vas deferens. E, Epithelium; L, lumen; M, muscle layer; Sc, Stereocilia; Sp, sperm. Scale bar 50 μm.

nerve fibers. Leydig cells are ovoid to irregular in shape, with dense spherical nuclei, and occur singly or in clusters within the interstitial tissue (Figure 5C).

Within the germinal epithelium, Sertoli cells are present as irregularly shaped somatic cells whose nuclei are located near the basal portion of the tubules (Figure 5D). Adjacent to these cells are the germ cells, which initiate spermatogenesis with mitotic proliferation of spermatogonia. Spermatogonia are spherical with dense diploid nuclei and, through mitosis, give rise to primary spermatocytes, which are slightly smaller and possess large ovoid nuclei; in some, chromosomal figures corresponding to subphases of meiotic prophase are evident. Primary spermatocytes undergo the first meiotic division to form secondary spermatocytes, which are smaller than primary spermatocytes. The second meiotic division produces spermatids, small spherical cells with dense nuclei. Through spermiogenesis, a maturation process, round spermatids transform into elongated spermatids and ultimately into spermatozoa, which are observed in clusters near the luminal border (Figure 5E).

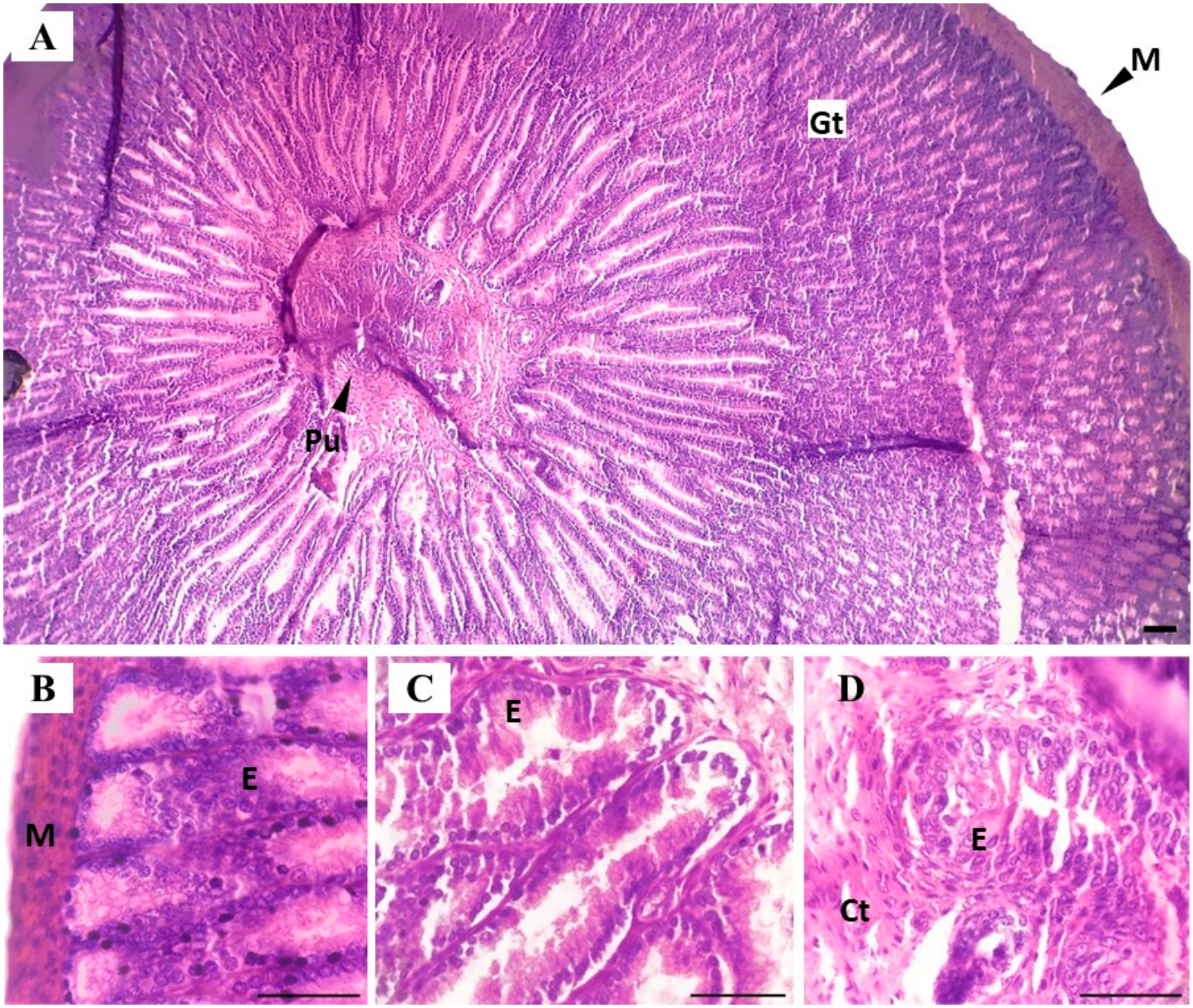

Figure 7. Prostate histology of *Marmosa robinsoni.* (A) Glandular tissue in the prostate; (B) Detail of the cortical region where the glandular tubules are arranged transversely; (C) Detail of the medullary region where the glandular tubules are arranged longitudinally; (D) Detail of the prostatic urethra. Ct, loose connective tissue; E, Epithelium; Gt, Glandular tissue; M, Muscle layer; Pu, Prostatic urethra. Scale bar 50 μm.

Testicular excurrent ducts. The *rete testis* consists of an interconnected network of channels that passively transport spermatozoa from seminiferous tubules toward the cranial region of the epididymis via the efferent ducts (*ductuli efferentes)* and epididymal ducts (*ductus epididymidis*). These channels are lined by simple cuboidal epithelium (Figure 6A).

Efferent ducts exhibit a simple columnar luminal epithelium with underlying connective tissue denser than that of the rete testis. Their lumen contains a translucent eosinophilic material in which scattered spermatozoa are observed (Figure 6B).

Epididymal ducts, embedded in loose connective tissue and surrounded by thin layers of smooth muscle fibers, are lined by simple columnar epithelium bearing conspicuous stereocilia. Spermatozoa are commonly observed within the lumen (Figure 6C).

The epididymis is formed by a highly coiled duct that extends along the external surface of the testis. Luminal epithelial morphology varies along its length, being simple columnar toward the head and body regions and simple cuboidal towards its tail portion. The epididymal epithelium is surrounded by a thin layer of vascularized connective tissue that adheres to the thicker connective tissue enclosing the testis.

Deferent ducts. Each deferent duct consists of a pseudostratified luminal epithelium surrounded by thick vascularized connective tissue and an outer thick layer of smooth muscle fibers (Figure 6D).

Prostate. The prostate is composed of glandular tissue arranged radially around the urethra and is surrounded by a layer of dense vascularized connective tissue and multiple layers of smooth muscle fibers. Glandular tubules are formed by secretory epithelial cells resting on a basal

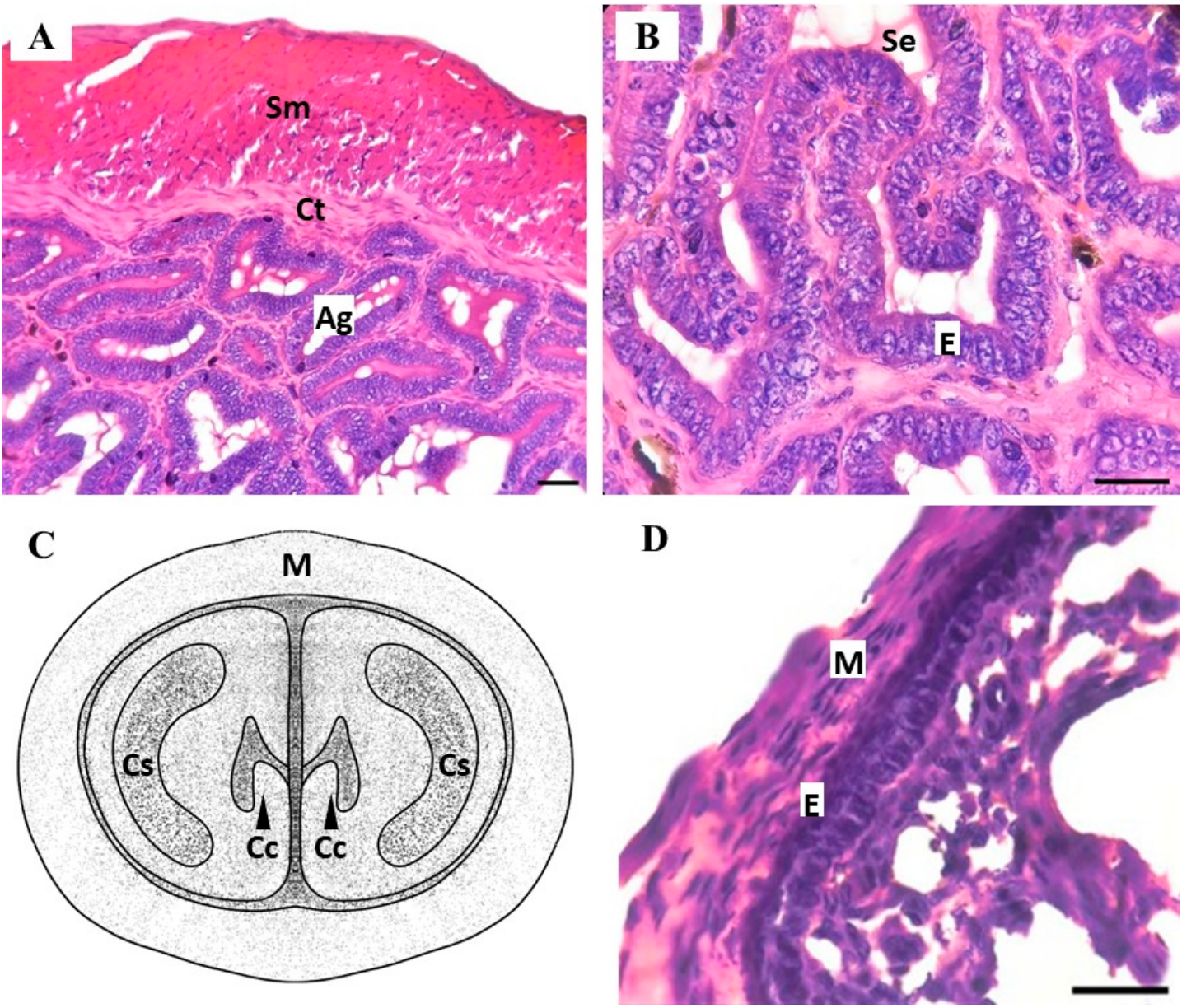

Figure 8. Histology of bulbourethral glands and penis of *Marmosa robinsoni*. (A) Cortex and part of the medulla of the bulbourethral gland; (B) Detail of the acinar glands in the medulla; (C) Illustration of the penis structure; (D) Detail of the peripheral area of the penis. Ag, Acinar glands; Cc, Corpus cavernosum; Cs, Corpus spongiosum; Ct, Connective tissue; E, Epithelium; M, Muscle layer; Se, Secretions; Sm, Striated muscle layer. Scale bar 25 μm.

lamina and are supported by loose connective tissue. In the cortical region of the prostatic urethra, tubules are arranged transversely, whereas toward the medullar part they become longitudinally oriented (Figure 7A). Glandular tubules in the cortical region exhibit simple cuboidal epithelium (Figure 7B); toward the medulla, the epithelium becomes columnar (Figure 7C) and changes into transitional epithelium lining the prostatic urethra (Figure 7D).

Bulbourethral glands. Bulbourethral glands are simple acinar glands externally surrounded by a thick layer of striated muscle and an additional layer of dense connective tissue that provides structural support (Figure 8A). Acini are lined by simple columnar epithelium and occasionally by transitional epithelium, with a prominent lumen that is often irregular due to evagination of the contorted sacs; abundant eosinophilic exocrine secretions are present within the lumen (Figure 8B).

Penis. The penis is composed of corpora cavernosa (*corpora cavernosa penis*) and corpora spongiosa (*corpus spongiosum penis*) forming the erectile tissue. These structures contain nerves, arteries, muscle fibers, and large blood-filled spaces, and each corpus is surrounded by dense connective tissue forming a thick *tunica albuginea* (Figure 8C). The corpus spongiosum encloses the urethra in more proximal sections. Surrounding these erectile bodies, a simple cuboidal epithelium is present, external to which lies a layer of smooth muscle fibers (Figure 8D).

Microscopic description of the female tract.

Ovaries. Ovaries are externally covered by simple squamous to stratified epithelium, immediately followed by a thin *tunica albuginea*. The presence or absence of an ovarian bursa could not be assessed from the ovarian histological sections. Folliculogenesis and follicular maturation occur within the cortical region; consequently,

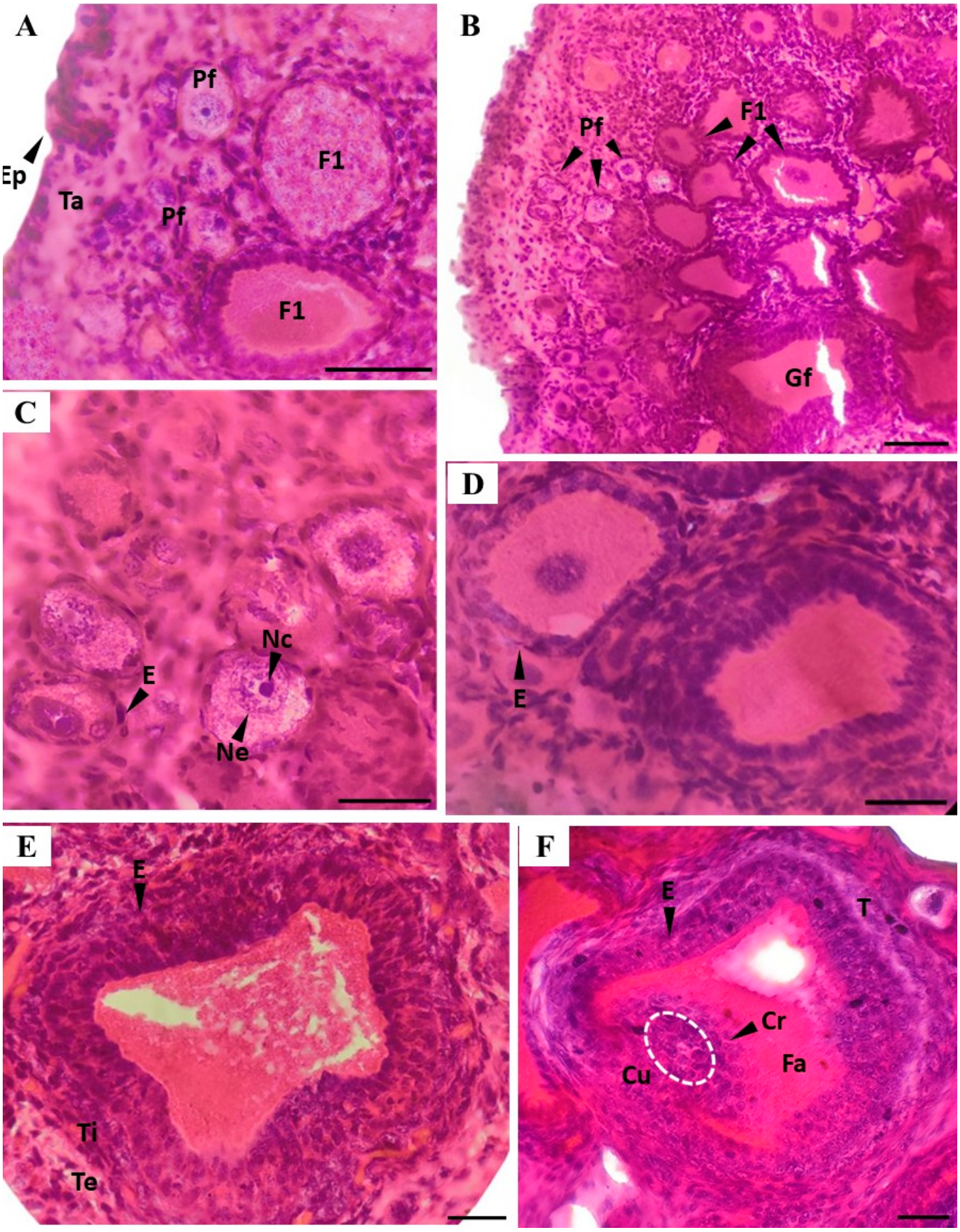

Figure 9. Ovarian histology of *Marmosa robinsoni*. (A) and (B) Follicular growth in the ovarian cortex; (C) Primordial follicles; (D) Primary follicles; (E) Growing follicle with increased follicular cell layers; (F) Graafian follicle. Cr, *Corona radiata*; Cu, Cumulus cells; Dotted oval, Oocyte; E, Epithelium; F1 Primary follicles; Fa, Follicular antrum; Gf, Growing follicle; Nc, Nucleolus; Ne, Nuclear envelope; Pf, Primordial follicles; T, Theca; Ta, *Tunica albuginea*; Te, Theca externa; Ti, Theca interna. Scale bar 25 *μm*.

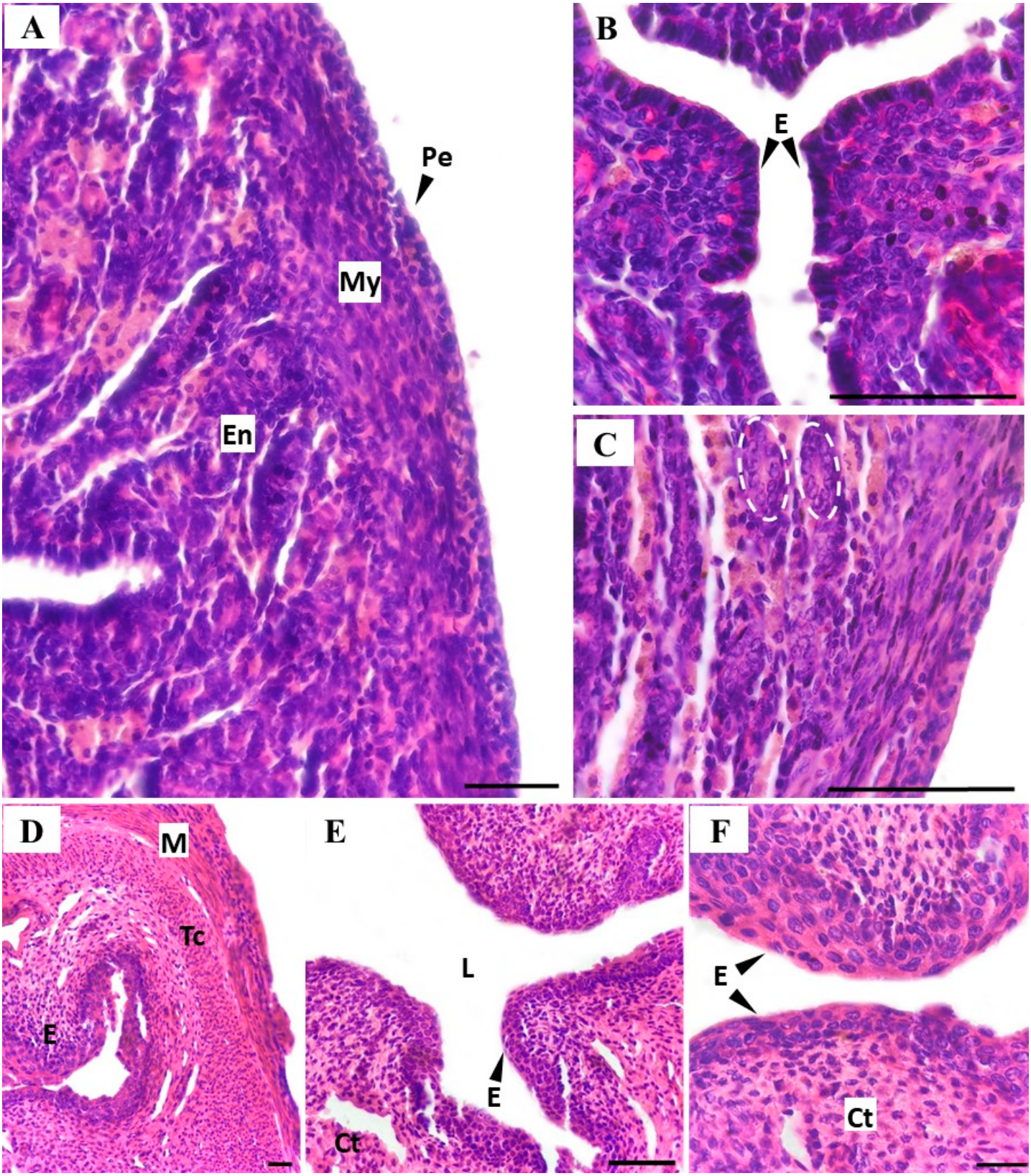

Figure 10. Histology of the uterus and urogenital sinus of *Marmosa robinsoni*. (A) Uterine layers; (B) Endometrial epithelium; (C) Endometrial glands; (D) Urogenital sinus; (E) Folds of the mucosa in the urogenital sinus; (F) Detail of the epithelium of the urogenital sinus. Ct, Connective tissue; Dotted lines, Glands in the endometrium with simple cuboidal epithelium; E, Epithelium; En, Endometrium; L, Lumen; M, Muscle layer; My, Myometrium; Pe, Perimetrium. Scale bars 25 µm.

follicles at multiple stages of development are present (Figure 9A, B). Each follicle contains an oocyte, a follicular epithelium composed of granulosa cells surrounding the oocyte, and an outer connective tissue layer forming the theca. Follicles are embedded within loose connective tissue, reflecting the compact organization of the ovary.

Primordial follicles are located near the *tunica albuginea* and are characterized by a relatively large, pale oocyte with

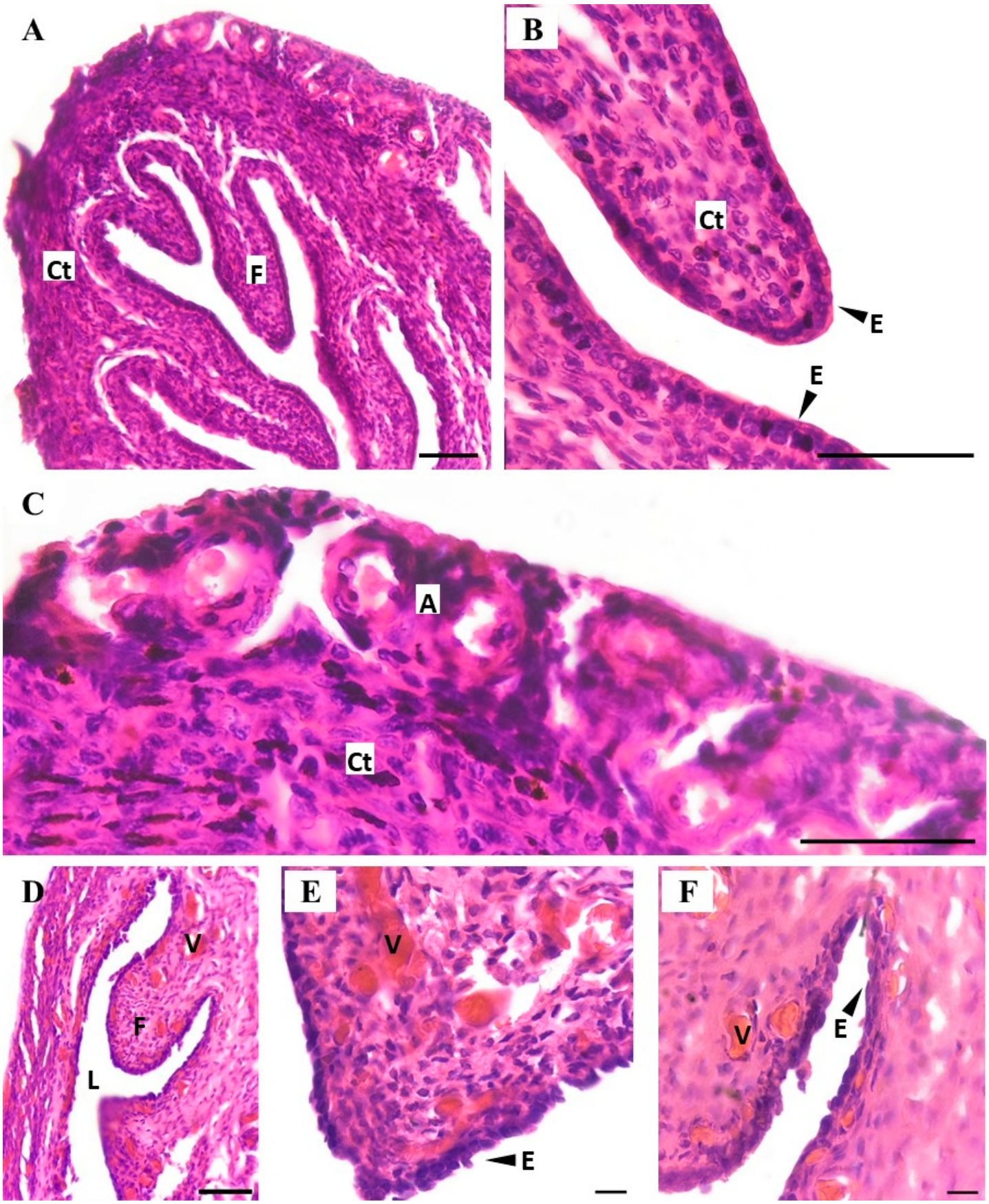

Figure 11. Histology of *Marmosa robinsoni* vaginae. (A) Folds in the mucosa of the lateral vagina; (B) Detail of the epithelium of the lateral vagina; (C) Detail of the external wall of the lateral vagina; (D) Medial vagina; (E) and (F) Detail of the luminal epithelium at the apex and at the base of the mucosal folds in the medial vagina. A, Arterioles; Ct, Connective tissue; F, Mucosal folds; L, Lumen; E, Epithelium; V, Blood vessels. Scale bars 50 μm for A, B, C, and D; and 25 μm for E and F.

a round nucleus, a conspicuous nuclear envelope, and a peripheral position with a single prominent nucleolus. The oocyte is surrounded by a simple squamous follicular epithelium constituting the granulosa cells (Figure 9C). With oocyte growth, the squamous granulosa epithelium becomes simple cuboidal and overlies a thin theca, forming primary follicles that increase in size (Figure 9D). Subsequent follicular growth is mediated primarily by an increase in the number of granulosa cell layers and thickening of the theca, producing follicles with two to three layers of stratified follicular epithelium and two distinct thecal layers: an inner layer of connective tissue with blood vessels and an outer layer of loose connective tissue (Figure 9E).

In large preovulatory follicles, an expanding follicular antrum displaces the oocyte toward the follicular periphery, forming a Graafian follicle. The oocyte is surrounded by granulosa cells forming the *corona radiata*, with cumulus cells at its base and several layers of follicular cells lining the follicular wall; externally, the thecal layers are present (Figure 9F). Notably, corpora lutea (*corpora lutea*) were not observed; therefore, none of the females examined were pregnant, nor was there evidence of lactation.

Uterus. The uteri are paired tubular organs composed of three tissue layers: endometrium (*endometrium*), myometrium (*myometrium*), and perimetrium (*perimetrium*) (Figure 10A). The innermost layer, the endometrium, is lined by simple columnar epithelium forming mucosal folds (Figure 10B). Its stroma contains numerous endometrial glands associated with the underlying connective tissue and lined by simple cuboidal epithelium (Figure 10C). Beneath the endometrium lies the myometrium, composed of layers of smooth muscle fibers, and externally the perimetrium, consisting of mesothelium and a thin layer of loose connective tissue.

Vaginae. The mucosa of the lateral vaginae forms numerous folds lined by non-keratinized stratified squamous epithelium and supported by dense connective tissue (Figure 11A, B). Numerous arterioles embedded within connective tissue are present in the peripheral region (Figure 11C).

The medial vagina or birth canal exhibits a mucosa with fewer folds lined by stratified epithelium (Figure 11D) that ranges from cuboidal to squamous, supported by dense, highly vascularized connective tissue (Figure 11E, F).

Urogenital sinus. The urogenital sinus is a continuous caudal canal in which the vaginal confluence lies cranial to the urethral opening and consists externally of a smooth muscle tunic overlying a thick layer of dense connective tissue. The mucosa forms broad folds lined by stratified squamous epithelium (Figure 10D–F).

Discussion

The present study represents the first contribution to the morphological and histological characterization of the reproductive system of *Marmosa robinsoni* and its gametogenic processes. Light microscopy analyses demonstrate that, in general terms, the gross and microscopic organization of the reproductive system—including organs, ducts, and both folliculogenesis and spermatogenesis in *M. robinsoni*—is consistent with descriptions reported for other marsupials, including didelphids (Nelsen and Maxwell 1942 in *Didelphis virginiana*; Barbour 1981 in *Lasiorhinus latifrons*; Fleming and Harder 1983 in *Didelphis virginiana*; Lyne and Hollis 1983 in *Isoodon macrourus* and *Perameles nasuta*; Cruz and Selwood 1993 in *Antechinus stuartii;* Richings *et al.* 2006 in *Macropus eugenii*; Cesario and Matheus 2008 in *Didelphis albiventris;* Gonçalves *et al.* 2009 in *Didelphis* sp.; de Barros *et al.* 2013 in *Didelphis* sp.; Lima *et al.* 2013 in *Gracilinanus microtarsus*; Khamas *et al.* 2014 in *Macropus giganteus giganteus;* Costa *et al.* 2015 in *Metachirus nudicaudatus*; Schimming *et al.* 2018 in *Didelphis albiventris*; Pagliarani *et al.* 2023 in *Phascolarctos cinereus*; Yllera *et al.* 2023 in *Petaurus breviceps*; Sepúlveda-Vásquez *et al.* 2025 in *Didelphis marsupialis*).

Male morphology and histology. The male reproductive tract of *M. robinsoni* is similar to that described for *Gracilinanus microtarsus* (Lima *et al.* 2013) and *Didelphis* sp. (de Barros *et al.* 2013) with respect to both organ position and overall organization. The histological organization of the testes and excurrent ducts follows the general pattern reported for other mammals and indicates that all three males examined were adults actively producing spermatozoa and possessed pigmented testes.

A notable difference, however, is the simple penile morphology observed in *M. robinsoni*, in contrast to species that develop a bifid penis glans (*glans penis*), such as *G. microtarsus* and *Didelphis* sp. It has been reported that in marsupial species such as *Gracilinanus agilis*, and in genera including *Marmosops*, *Micoureus*, *Thylamys*, and *Metachirus*, the glans is initially simple in juvenile individuals and gradually becomes bifid as animals mature (Dixson 2021). In the present study, all examined individuals of *M. robinsoni* were adults, with large testes and advanced spermatogenesis, confirming that the absence of bifurcation in the penis glans is a species-specific trait. This condition is consistent with the plesiomorphic state reported for other marsupials such as *Marmosa mexicana*, opossums, kangaroos, and wallabies (Dixson 2021). It could be hypothesized that a bifid glans would be functionally compatible with the presence of lateral vaginae, potentially facilitating sperm deposition during copulation. However, studies in wallabies have demonstrated that spermatozoa travel effectively through the lateral vaginae even in the absence of a bifid glans (Dixson 2021).

Additional differences were observed in the arrangement of the bulbourethral glands, with two pairs present in *M. robinsoni*, compared with three pairs described for other didelphids such as *Didelphis* sp. (de Barros *et al.* 2013) and *G. microtarsus* (Lima *et al.* 2013). Variation in the number of bulbourethral gland pairs appears to be species-specific. Studies in at least eight Australian marsupial species and in 22 South American didelphid species report the presence

of two to three pairs of these glands (Rodger and Hughes 1973; Nogueira *et al.* 2004).

Testicular morphology in *M. robinsoni* is characterized by a pigmented *tunica vaginalis*, conferring an intense bluish coloration similar to that reported for *G. microtarsus* and adult individuals of *Didelphis* sp., highlighting that dark testicular coloration is an attribute of sexually mature individuals (de Barros *et al.* 2013; Lima *et al.* 2013). Additionally, Nogueira *et al.* (2004) found that 21 of 22 South American didelphid species examined exhibited this distinctive coloration. Many descriptions of pigmented testes, however, do not specify the precise tissue location of the pigment. In many marsupials, pigmentation is localized to the *tunica vaginalis* rather than the *tunica albuginea*, which differs from most placental mammals. Testicular pigmentation in mammals may therefore occur in the outermost layer (*tunica vaginalis*) as in some marsupials, in the *tunica albuginea* as in some eutherians, or in both, but not in the scrotal skin (Finkel 1945; Biggers 1966; Heddle and Guiler 1970; de Barros *et al.* 2013). The functional significance of this pigmentation may be related to local thermal regulation, as darker tissues absorb more incident radiation (Biggers 1966), although this relationship remains inconclusive given interspecific differences in climatic conditions.

Female morphology and histology. This study reports the presence of a dark pigmented patch, visible internally, located on the ventral body wall beneath the mammary region in adult females of *Marmosa robinsoni*. To our knowledge, the occurrence of a pigmented patch underlying the mammary glands has not been previously documented in marsupials, and therefore this represents the first report of such a feature. The pigmented area is observed only during dissection and is not externally visible, indicating that it is restricted to deeper tissues beneath the skin. Moreover, the strict alignment of the pigmented patch with the entire nipple series suggests an association with mammary-related structures.

Pigmentation in non-cutaneous connective tissues of marsupials has been documented in other contexts, such as the *tunica vaginalis* of the testes, as discussed above. This characteristic may reflect a broader tendency for ectopic pigment deposition in tissues associated with ectoderm–mesenchyme interactions within hormonally responsive glandular fields. The submammary pigmented patch observed in *M. robinsoni* may represent a comparable phenomenon at the embryological and tissue levels, without implying direct homology.

The taxonomic distribution and biological significance of this trait remain uncertain, and in the absence of histological confirmation in the present study, we refrain from assigning a functional role or diagnostic value to this structure. Accordingly, we interpret the submammary pigmented patch as a potential anatomical variation possibly associated with development of the mammary line. Additional comparative and histological studies are required to evaluate its tissue composition and relevance within *Marmosa* and related didelphid taxa.

The morphology and histology of the ovaries, oviducts, and uterus in *Marmosa robinsoni* are consistent with the general pattern described for other marsupials, including didelphids and diprotodontians (Harder *et al.* 1993; Regli and Kress 2002; Pagliarani *et al.* 2023; Sepúlveda-Vásquez *et al.* 2025).

Folliculogenesis follows the pattern described for mammals in general and for marsupials such as *Isoodon macrourus*, and *Perameles nasuta* (Lyne and Hollis 1983); *Didelphis albiventris* (Cesario and Matheus 2008), and in *Phascolarctos cinereus* (Pagliarani *et al.* 2023). Likewise, ovarian histology and that of the remaining ducts and organs conform to the general mammalian model. All our adult females exhibited follicles at different stages of development, from primordial to antral follicles, the latter representing the most advanced stage observed. The absence of postovulatory structures indicates that these females were in the follicular growth phase. The juvenile female exhibited only primordial and primary follicles and lacked prominent mammary glands and the pigmented submammary patch.

In the vaginal complex, a distinct cul-de-sac comparable to that described in *Didelphis* spp. (Nelsen and Maxwell 1942; Harder *et al.* 1993; Sepúlveda-Vásquez *et al.* 2025) was not clearly recognized in the present material. However, the cranial portions of the lateral vaginae were incompletely represented in our histological sections, precluding a definitive assessment of its presence. On the other hand, the proposed separation of a vaginal sinus from the urogenital sinus by Sepúlveda-Vasquez *et al.* (2025) for *Didelphis marsupialis* in our species is topological rather than morphological, as both regions form part of a continuous canal lacking a conspicuous macroscopic boundary. The distinction is because the urethra joins the common tract, caudal to the confluence of the vaginal ducts.

Vestibular (Bartholin-like) glands have been described in only a few marsupials, including the koala (*Phascolarctos cinereus*) (Pagliarani *et al.* 2023). No such glands were evident in *Marmosa robinsoni*, nor have they been reported in most recent anatomical and histological descriptions of didelphids (Regli and Kress 2002; Schimming *et al.* 2018; Sepúlveda-Vásquez *et al.* 2025), suggesting that they may be poorly developed, inconspicuous, or simply overlooked in many species.

Histologically, the predominance of stratified squamous epithelium in the lateral vaginae is consistent with that reported for *Didelphis albiventris* (Schimming *et al.* 2018), the koala, *Phascolarctos cinereus* (Pagliarani *et al.* 2023), and other marsupials. In contrast, the median vagina (birth canal) of *M. robinsoni* exhibited epithelial variation ranging from cuboidal to squamous stratified, like observations by Nelsen and Maxwell (1942). Epithelial differentiation within the vaginal complex may vary according to reproductive stage in *Monodelphis domestica* (Regli and Kress 2002) and

across reproductive phases in the koala (Pagliarani *et al.* 2023), this differentiation mainly involves differences in epithelial thickness, stratification, and cellular differentiation. In the present study, all adult females examined were in the same reproductive stage, therefore, the epithelial variation observed in the median vagina is unlikely to be associated with reproductive stage and more likely reflects intrinsic regional differentiation within the birth canal.

In marsupials, as in eutherian mammals, the vaginal epithelium undergoes a series of well-defined, hormonally regulated morphological changes (Hinds *et al.* 1996), suggesting a conserved hormonal regulation of the lower female reproductive tract across Theria. The evaluation of these changes can be used to determine the estrous cycle of individuals (e.g., Godfrey 1975 in a captive population of *Marmosa robinsoni*; Crawford *et al.* 1999 in *Trichosurus vulpecula*; Regli and Kress 2002 in *Monodelphis domestica;* Pagliarani *et al.* 2023 in *Phascolarctos cinereus*). In the present study, based on the characteristics of a proliferative vaginal luminal epithelium that is not yet markedly thickened and lacks evidence of keratinization, we infer that all adult females examined were in the follicular phase of the ovarian cycle. This interpretation is supported by the presence of growing tertiary ovarian follicles with developing antra, absence of evidence of recent ovulation, and lack of *corpora lutea*.

Bradshaw and Bradshaw (2011) proposed that, in marsupials, unlike in eutherians, formation of the corpus luteum does not always occur immediately after ovulation. In species with short gestation periods (11–15 days), the corpus luteum generally reaches its maximum size during the first week after ovulation, whereas species with longer gestations are characterized by slower-developing and more persistent corpora lutea. The absence of *corpora lutea* or *corpora albicantia* in the ovaries of *M. robinsoni*, a species with a short gestation period (approximately 14 days), may therefore be explained by the fact that none of the females examined was pregnant and by the potentially brief lifespan of this structure in species with this reproductive strategy.

Finaly, the secondary sexual characteristics observed (pigmented scrotal testes in males and developed mammary glands in females) were directly related to individual maturity and therefore constitute external morphological features useful for studies of natural history and for assessing sex and reproductive status in this species.

In conclusion, this study provides the first comprehensive macroscopic and microscopic characterization of the male and female reproductive systems of *Marmosa robinsoni*, establishing an anatomical and histological framework for the species. The overall organization of organs, ducts, and gametogenic processes conforms to the general marsupial pattern, while notable species-specific traits—such as the simple, non-bifid penis, the arrangement of bulbourethral glands, and the presence of an internal submammary pigmented patch in females—expand current knowledge of morphological diversity within Didelphidae.

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Declaration of Artificial Intelligence use

AI was employed to assist with grammar, spelling, clarity, and overall readability of the English language. No AI tools were used to generate original ideas, content, analysis, or conclusions. All intellectual content, interpretations, and arguments presented in this work are entirely our own.

Author contributions

Mayra A. Galezo-Suárez, a student trained through this work, carried out the fieldwork and performed a substantial portion of the laboratory analyses. Nathaly Hernández-Díaz supervised the work and provided training in laboratory and analysis procedures. Víctor H. Serrano-Cardozo and Martha P. Ramírez-Pinilla were responsible for the overall project, including its conception, design, and completion. All authors contributed to the writing, review, and revision of the manuscript, and have read and approved the final version.

Supplementary data

Table SD1. Composition of baits used in the field phase.

Table SD2. Total length measurements of the individuals captured and entered the Mammalogy collection of the Natural History Museum of the Industrial University of Santander.

Figure SD3. Individuals captured in the study area and unrelated to the object of study. *Nephelomys sp.* (A y D) y *Rhipidomys sp.* (B y C).

Figure SD4. *Marmosa robinsoni* male, UIS-MHN-M-2201.

Figure SD5. Exposure of the abdominal cavity of *Marmosa robinsoni* in males (A) and females (B). B, bladder; Dp, Submammary pigmented dermal patch; I, Intestine; L, Liver; S, Sternum; T, Testes; X, Xiphoid process. Scale bar 20 mm.

Figure SD6. Digestive system (A) and liver (B) of a female *Marmosa robinsoni*. An, Anus; Ce, Cecum; Cl, Central lobe of the liver; Co, Colon; Du, Duodenum; I, Ilium; J, Jejunum; Ll, Left lobe of the liver; Rl, Right lobe of the liver; S, Stomach. Scale bar 10 mm.

Literature cited

American Veterinary Medical Association [AVMA]. 2020. *Guidelines for the Euthanasia of Animals: 2020 edition*. Illinois (USA): AVMA.

Barbour RA. 1981. Histology and histochemistry of the accessory reproductive glands in the male hairy-nosed wombat (*Lasiorhinus latifrons*). *Histochemistry* 72:133–148. https://doi.org/10.1007/BF00496788.

Biggers JD. 1966. Reproduction in male marsupials. In: Rowlands IW, editor. *Comparative biology of reproduction in mammals*. New York (USA): Academic Press; p. 251–280.

Bradshaw FJ, and Bradshaw D. 2011. Progesterone and

reproduction in marsupials: a review. *General and Comparative Endocrinology* 170:18–40. https://doi.org/10.1016/j.ygcen.2010.07.015

Cadena Bohórquez JG. 2016. *Descripción histológica del sistema reproductor de la hembra de chucha común (Didelphis marsupialis)* [Bachelor's Thesis]. [Villavicencio (COL)]: Universidad de los Llanos.

Ceballos G, Ortega B, Sühring S, Domínguez Y, and Zarza H. 2002. Mamíferos de Venezuela. In: Ceballos G, and Simonetti JA, editors. *Diversidad y conservación de los mamíferos neotropicales*. Mexico City (MEX): Comisión Nacional para el Conocimiento y Uso de la Biodiversidad.

Costa SF, Nogueira JC, Soares BA, Ambrósio NA, Chaves AS, de Melo LQ, *et al*. 2015. Morfologia do escroto, do testículo e das vías espermáticas de *Metachirus nudicaudatus* (Geoffroy, 1803), Didelphidae-Marsupialia. *Pesquisa Veterinária Brasileira* 35:69–83. https://doi.org/10.1590/S0100-736X2015001300012

Crawford JL, McLeod BJ, and Hurst PR. 1999. Cyclical changes in epithelial cells of the vaginal cul-de-sac of brushtail possums (*Trichosurus vulpecula*). *The Anatomical Record* 254:307–321. https://doi.org/10.1002/(SICI)1097-0185(19990301)254:3%3C307::AID-AR1%3E3.0.CO;2-U

Cruz YP, and Selwood L. 1993. Uterine histology of the dasyurid marsupial, *Antechinus stuartii*: relationship with differentiation of the embryo. *Journal of Reproduction and Fertility* 99:237–242. https://doi.org/10.1530/jrf.0.0990237

Cuartas-Calle C, and Muñoz J. 2003. *Marsupiales, cenoléstidos e insectívoros de Colombia*. Medellín (COL): Editorial Universidad de Antioquia.

Cesario MD, and Matheus SMM. 2008. Structural and ultrastructural aspects of folliculogenesis in *Didelphis albiventris*, the South-American opossum. *International Journal of Morphology* 26:113–109. http://dx.doi.org/10.4067/S0717-95022008000100019

de Barros MA, Panattoni Martins JF, Samoto VY, Oliveira VC, Gonçalves N, Celina CA, *et al*. 2013. Marsupial morphology of reproduction: South American opossum male model. *Microscopy Research and Technique* 76:388–397. https://doi.org/10.1002/jemt.22178

Dixson AF. 2021. *Mammalian sexuality: the act of mating and the evolution of reproduction*. Cambridge University Press.

Finkel MP. 1945. The relation of sex hormones to pigmentation and to testis descent in the opossum and ground squirrel. *The American Journal of Anatomy* 76:93–151.

Fleming MW, and Harder JD. 1983. Luteal and follicular populations in the ovary of the opossum (*Didelphis virginiana*) after ovulation. *Journal of Reproduction and Fertility* 67:29–34. https://doi.org/10.1530/jrf.0.0670029

Global Biodiversity Information Facility [GBIF]. 2022. *Marmosa (Exulomarmosa) robinsoni* Bangs, 1898. Sistema Global de Información sobre Biodiversidad. [Accessed July 20th, 2023] https://www.gbif.org/es/species/2439862

Godfrey GK. 1975. A study of oestrus and fecundity in a laboratory colony of mouse opossums (*Marmosa robinsoni*). *Journal of Zoology* 175:541–555. https://doi.org/10.1111/j.1469-7998.1975.tb01416.x

Gonçalves NN, Maçanares CAF, Miglino MA, Samoto VY, Martins DDS, Ambrósio CE, *et al*. 2009. Aspectos morfológicos dos *órgãos* genitais femininos do gambá (*Didelphis sp*.). *Brazilian Journal of Veterinary Research and Animal Science* 46:332–338. https://doi.org/10.11606/issn.1678-4456.bjvras.2009.26782

Gutiérrez EE, Anderson RP, Voss RS, Ochoa-G J, Aguilera M, and Jansa SA. 2014. Phylogeography of *Marmosa robinsoni*: insights into the biogeography of dry forests in northern South America. *Journal of Mammalogy* 95:1175–1188. https://doi.org/10.1644/14-MAMM-A-069

Hall ER. 1962. Collecting and preparing study specimens of vertebrates. *Miscellaneous Publications of the University of Kansas Museum of Natural History* 30:1–46.

Harder JD, Stonerook MJ, and Pondy J. 1993. Gestation and placentation in two New World opossums: *Didelphis virginiana* and *Monodelphis domestica*. *Journal of Experimental Zoology* 266:463–79. https://doi.org/10.1002/jez.1402660511

Heddle RWL, and Guiler ER. 1970. The form and function of the testicular rete mirabile of marsupials. *Comparative Biochemistry and Physiology* 35:415–425. https://doi.org/10.1016/0010-406X(70)90605-5

Hinds LA, Fletcher TP, and Rodger JC. 1996. Hormones of oestrus and ovulation and their manipulation in marsupials. *Reproduction, Fertility and Development* 8:661–672. https://doi.org/10.1071/RD9960661

Khamas W, Al-Tikriti M, Albayati M, Tkalcic S, and Eng C. 2014. Histological description of the testis, epididymis and ductus deferens of the Northern great grey kangaroo (*Macropus giganteus giganteus*). *Journal of Cytology and Histology* 5:1–6. https://doi:10.4172/2157-7099.1000287

Lima JMN, Santos AC, Viana DC, Bertassoli BM, Lobo LM, Oliveira VC, *et al*. 2013. Morphological study of the male genital organs of *Gracilinanus microtarsus*. *Brazilian Journal of Veterinary Research and Animal Science* 50:447–456. https://doi.org/10.11606/issn.1678-4456.v50i6p447-456

López-Fuster M, Pérez-Hernández R, Ventura J, and Salazar M. 2000. Effect of environment on skull-size variation in *Marmosa robinsoni* in Venezuela. *Journal of Mammalogy* 81:829–837. https://doi.org/10.1644/1545-1542(2000)081%3C0829:EOEOSS%3E2.3.CO;2

Luna LG. 1968. *Manual of histology staining methods of the Armed Forces Institute of Pathology*. New York (USA): McGraw Hill.

Lyne AG, and Hollis DE. 1983. Observations on Graafian follicles and their oocytes during lactation and after the removal of pouch young in the marsupials *Isoodon macrourus* and *Perameles nasuta*. *American Journal of Anatomy* 166:41–61. https://doi.org/10.1002/aja.1001660104

Mills JN, Childs JE, Ksiazek TG, Peters CJ, Velleca WM. 1998. *Métodos para trampeo y muestreo de pequeños mamíferos para estudios virológicos*. Washington (USA): Centros para el Control y la Prevención de Enfermedades.

Ministerio del Ambiente [MINAM]. 2015. *Guía de inventario de la fauna silvestre*. Resolución Ministerial N° 057-2015-MINAM. Lima (PER): Ministerio del Ambiente.

Morales-Jiménez AL, Sánchez F, Poveda K, and Cadena A. 2004. Mamíferos terrestres y voladores de Colombia: guía de campo. Bogotá (COL): Universidad Nacional de Colombia.

Nelsen OE, and Maxwell N. 1942. The structure and function of the urogenital region in the female opossum compared with the same region in other marsupials. *Journal of Morphology* 71:463–491. https://doi.org/10.1002/jmor.1050710304

Nogueira JC, Castro ACS, Câmara EVC, and *Câmara BGO. 2004*. Morphology of the male genital system of *Chironectes minimus* and comparison to other didelphid marsupials. *Journal of Mammalogy* 85:834–841. https://doi.org/10.1644/207

O'Connell MA. 1983. *Marmosa robinsoni. Mammalian Species* 203:1–6. https://doi.org/10.2307/3504031

Padilla-Rivera O. 2018. Abundancia de *Marmosa xerophila* y *Marmosa robinsoni* (Didelphimorphia: Didelphidae) en la mina El Cerrejón, La Guajira, Colombia. *Mammalogy Notes* 5:1–2. https://doi.org/10.47603/manovol5n1.26-30

Pagliarani S, Palmieri C, McGowan M, Carrick F, Boyd J, and Johnston SD. 2023. Anatomy of the female koala reproductive tract. *Biology* 12:1445. https://doi.org/10.3390/biology12111445

Patiño J. 2022. Riqueza y variación morfológica de los géneros *Marmosa* y *Marmosops* (Didelphidae) en valles interandinos de Colombia [Bachelor's Thesis]. [Caldas (COL)]: Universidad de Caldas.

Pérez-Hernández R. 2016. *Marmosa robinsoni*. The IUCN Red List of Threatened Species 2016:e.T40506A22174162. [Accesed July 18th, 2024] https://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T40506A22174162.en

Rangel JO, Rivera A, Rincón H, Arellano JE, Carvajal S, Ávila M, *et al.* 2012. Bosque del Agüil (Aguachica-Cesar), biodiversidad, educación ambiental y conservación. In: Rangel JO, editor. *Colombia Diversidad Biótica*. *Publicación Especial No. 4*. Bogotá (COL): Instituto de Ciencias Naturales.

Regli C, and Kress A. 2002. Changes in the epithelium of the vaginal complex during the estrous cycle of the marsupial *Monodelphis domestica*. *Cells Tissues Organs* 172:276–296. https://doi.org/10.1159/000067197

Reig O. 1968. The chromosomes of the didelphiid marsupial *Marmosa robinsoni* bangs. *Experientia* 24:185–186. https://doi.org/10.1007/bf02146978

Renfree M, and Shaw G. 2001. Reproduction in monotremes and marsupials. In: Renfree M, editor. *Encyclopedia of Life Sciences*. Chichester (UK): John Wiley & Sons, Ltd.

Richings NM, Shaw G, Temple-Smith PD, and Renfree MB. 2006. Growth and histology of ovarian follicles after cold storage in the tammar wallaby. *Reproduction, Fertility and Development* 18:677–688. https://doi.org/10.1071/RD06007

Rodger JC, and Hughes RL. 1973. Studies of the accessory glands of male marsupials. *Australian Journal of Zoology* 21:303–320. https://doi.org/10.1071/ZO9730303

Romero-Almaráz M, Sánchez-Hernández C, García-Estrada C, and Owen R. 2007. *Mamíferos pequeños: manual de técnicas de captura, preparación, preservación y estudio*. Mexico City (MEX): Instituto de Biología, UNAM.

Rossi RV, Voss RS, and Lunde DP. 2010. A revision of the didelphid marsupial genus *Marmosa*. Part 1. The species in Tate's "Mexicana" and "Mitis" sections and other closely related forms. *Bulletin of the American Museum of Natural History* 334:1–83. https://doi.org/10.1206/334.1

Rueda MC, Ramírez G, and Osorio J. 2013. Aproximación a la biología de la zarigüeya común (*Didelphis marsupialis*). *Boletín Científico Museo de Historia Natural* 17:141–153.

Schimming BC, Cesario MD, and Matheus SMM. 2018. Morphology of the vaginal complex in the white-eared opossum (*Didelphis albiventris*): gross anatomy and light microscopy. *Anatomia, Histologia, Embryologia* 47:566–572. https://doi.org/10.1111/ahe.12398

Schneider CA, Rasband WS, and Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9:671–675. https://doi.org/10.1038/nmeth.2089

Sepúlveda-Vásquez A, Ceballos CP, and Tamayo-Arango LJ. 2025. Reproductive tract and pouch anatomical variability across the reproductive phases in female common opossum (*Didelphis marsupialis* Linnaeus, 1758). *PLoS One* 20:e0334040. https:// doi.org/10.1371/journal.pone.0334040

Sikes RS, Thompson TA, and Bryan JA. 2019. American Society of Mammalogists: raising the standards for ethical and appropriate oversight of wildlife research. *Journal of Mammalogy* 100:763–773. https://doi. org/10. 1093/ jmamm al/ gyz019

Voss R. 2013. *Marmosa robinsoni*. Animal Diversity Web. [Accessed June 24th, 2025] https://animaldiversity.org/accounts/Marmosa_robinsoni/

Voss R, and Jansa S. 2009. Phylogenetic relationships and classification of didelphid marsupials, an extant radiation of New World metatherian mammals. *Bulletin of the American Museum of Natural History* 322:1–177. https://doi.org/10.1206/322.1

Voss R, and Jansa S. 2021. *Opossums: an adaptive radiation of New World marsupials*. Maryland (USA): Johns Hopkins University Press.

Yllera MM, Alonso-Peñarando D, Lombardero M. 2023. Gross anatomy of the female reproductive system of sugar gliders (*Petaurus breviceps*). *Animals* 13:2377. https://doi.org/10.3390/ani13142377

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