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The Unique Penile Morphology of the Short-Beaked Echidna, *Tachyglossus aculeatus*

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Evolution · Penile morphology · Echidna · Monotreme · Cloaca

Abstract

Monotremes diverged from therian mammal ancestors approximately 184 million years ago and have a number of novel reproductive characteristics. One in particular is their penile morphology. There are differences between echidna and platypus phalluses, but both are somewhat similar in structure to the reptilian phallus. The echidna penis consists of 4 rosette glans, each of which contains a termination of the quadrifurcate urethra, but it appears that only 2 of the 4 glans become erect at any one time. Despite this, only a few historical references describe the structure of the echidna penis and none provides an explanation for the mechanisms of unilateral ejaculation. This study confirmed that the echidna penis contains many of the same overall structures and morphology as other mammalian penises and a number of features homologous with reptiles. The corpus cavernosum is well supplied with blood, extends up to the base of the glans penis and is primarily responsible for erection. However, the echidna possesses 2 distinct corpora spongiosa separated by a septum, each of which surround the urethra only distal to the initial urethral bifurcation in the glans penis. Together with the bifurcation of the main penile artery, this provides a mechanism by which blood flow could be directed to only one corpus spongiosum at a time to maintain an open urethra that supplies 2 of the 4 glans to facilitate unilateral ejaculation.

Introduction

In mammals, the male copulatory apparatus has evolved rapidly even among closely related species, and can vary in morphology, surface epithelial features, and internal elements [Owen, 1868; Woolley and Webb, 1977; Gredler et al., 2014; Kelly, 2016]. Many of these differences are hypothesised to be due to post-copulatory sexual selection, influenced by the physical interaction of male and female genital tissues during copulation [Brennan, 2016]. There are numerous mechanisms for penis retraction, with associated variation in the muscles that control its position, and the biomechanics required to transform it to an erectile state [Kelly, 2016].

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In therian mammals, the penis contains a centrally located urethra, surrounded by the corpus spongiosum and ventral to this is the erectile corpus cavernosum. Many mammals possess a baculum (os penis) [Schultz et al., 2016], a preputial sheath and, if the penis is stored internally, a retractor muscle [Bassett, 1961]. Some marsupials have a bifurcated glans penis with a levator muscle and a split urethra passing through each glans, and dasyurids have a wide variety of erectile bodies [Owen, 1868; Biggers, 1966; Woolley and Webb, 1977; Tyndale-Biscoe and Renfree, 1987]. In the bilby, the glans is split into two, and the urethra bifurcates just proximal to the split of the glans and this gives rise to 2 separate urethrae [Owen, 1868; Johnston et al., 2010].

Monotremes, which diverged from therian mammals around 184 million years ago [Cúneo et al., 2013], have a number of reproductive characteristics that differ from the majority of therian mammals. They are the only mammalian order to retain the cloaca into adulthood; they are testicond with the testes being retained in the abdominal cavity, rather than descending to lie externally either in a scrotum or in the inguinal canal; their ejaculate contains bundles of sperm that swim cooperatively; and the monotreme penis is used purely as a reproductive organ, unlike therian mammals whose penile urethra is used for transport of both urine and semen [Owen, 1868; Griffiths, 1968; Johnston et al., 2007a].

There are 5 extant species of monotremes, the platypus (*Ornithorhynchus anatinus*), the short-beaked echidna (*Tachyglossus aculeatus*), and 3 species of long-beaked echidna (*Zaglossus bartoni*, *Zaglossus attenboroughi*, and *Zaglossus bruijnii*), the latter 3 endangered. In adult males of both the platypus and short-beaked echidna, the penis retracts internally into a preputial sheath [Griffiths, 1968]. However, the platypus and short-beaked echidna have different penile morphology. Whilst the platypus penis is covered with multiple, distinct epidermal spines and has a bifurcated urethra that exits from epithelial foliate papillae [Home, 1802a; Owen, 1868; Disselhorst, 1904; Temple-Smith, 1973], the short-beaked echidna penis has a few very small epidermal spines but is mostly smooth with a quadrifurcate urethra that exits from 4 orifices surrounded by concentric circles of papillae [Home, 1802b; Owen, 1868; Disselhorst, 1904]. Upon erection of the echidna penis, the engorgement forces the head of the penis forward from its retracted location above the cloaca to pass briefly through the lumen of the cloaca via a sphincter before exiting through the cloacal aperture [Johnston et al., 2006b]. It appears that the 4 openings in the echidna are used unilaterally where the use of each lateral pair apparently alternates between successive copulations [Johnston et al., 2007b]. However, since this observation is from a single male echidna, it is unknown if this occurs in all male echidnas or how it is controlled.

Unlike in eutherians and marsupials, the ureters in monotremes do not empty directly into the bladder. Instead, they enter the urogenital sinus at the base of the bladder. The extensive musculature surrounding the bladder controls the urine movement so that urine is stored in the bladder [Keibel, 1904; Griffiths, 1968]. The vas deferens in the echidna enters the urogenital sinus just distal to where the ureters empty urine from the kidneys. Thus the urogenital sinus conveys both urine (from the bladder) to the cloaca and semen (from the vas deferens) to the penis and is connected to the penile urethra by a small orifice at the base of the penis. However, it is unknown how semen is directed at copulation into the penile urethra rather than following the same path as the urine directly into the cloaca. Three mechanisms for this have been proposed. The first is that it is controlled via the combined action of the retractor penis muscle with the cloacal sphincter [Owen, 1868; Disselhorst, 1904]. The second is that the enlargement of the penis at erection blocks the cloacal entry and forces the semen to the penile urethra [Griffiths, 1968; Johnston et al., 2007a]. Thirdly, when the penis is manually pulled out of the cloaca, it kinks the urogenital canal distal to the opening into the penile urethra, which might prevent sperm from entering the cloaca and therefore force them out via the penile urethra [Boas, 1891].

The apparent unilateral alternation of ejaculation from 2 of the 4 urethral openings, and the exclusive use of the penis in the echidna for conveying sperm, have been compared to squamate hemipenes [Owen, 1868; Boas, 1891; Gerhardt, 1909; Johnston et al., 2007b]. Squamates (snakes and lizards) possess hemipenes, dual bifurcate penile structures used exclusively for reproduction. In many squamate species, only one hemipenis is used during mating at any time, and these alternate between copulations, similar to that reported for the echidna [Dowling and Savage, 1960; Johnston et al., 2007b; Gredler et al., 2014; Leal and Cohn, 2014]. However, in all reptiles, semen is transported along the phallus by means of an open groove-like structure called the spermatic sulcus, rather than by an enclosed urethra [Gredler et al., 2014]. The extent to which the echidna model of ejaculation resembles that of the squamate hemipenes is unknown.

The echidna and platypus overall penis structures are quite distinct from that of other mammals but appear to have some similarity to reptiles in their penile function.

However, in the echidna there are no detailed morphological descriptions or information on the mechanisms controlling penile erection and retraction or how ejaculation might occur unilaterally. This study addresses these questions.

Materials and Methods

Animals

Adult male echidna reproductive tracts ($n = 5$) were collected opportunistically from injured animals brought into the Currumbin Wildlife Hospital (SE Queensland) that required euthanasia for animal welfare reasons; no echidnas were killed for this research. Additional euthanised males ($n = 2$) were used for the MR images and the location of the penile retractor muscles in situ as detailed below.

Histology

After euthanasia, the reproductive tract was removed and the penis was dissected out and fixed in 4% PFA overnight, washed twice in 1× PBS and stored in 70% ethanol ($n = 4$). Due to their size, penises for histology were further dissected before processing. Penises were initially dissected into evenly distributed quarters with medial incisions from proximal to distal. Two of the penises were further bisected before processing. Samples were then processed as per standard histology procedures, embedded in paraffin and serially sectioned at 7 μm. Every second slide was stained with haematoxylin and eosin (H&E) for histological examination as per standard procedures, and representative slides were stained with Mallory's trichome to visualise connective tissues, primarily collagen. Briefly, for Mallory's trichome, slides were deparaffinized and rehydrated through a series of 100, 95, and 70% ethanol, then washed in distilled H_2O (dH_2O). Slides were first stained in haematoxylin for 5 min, rinsed in running tap water for 3 min, then washed in dH_2O. Slides were then stained in Biebrich scarlet-acid fuchsin solution for 10 min, washed in dH_2O and differentiated in phosphomolybdic-phosphotungstic acid solution for 15 min. Slides were then immediately transferred to aniline blue solution for 5 min, rinsed briefly in dH_2O, differentiated in 1% acetic acid solution for 3 min, and washed in dH_2O before being dehydrated in 95% ethanol and 2× 100% ethanol, cleared in histolene, and mounted.

Micro-CT

One additional penis fixed whole in 10% neutral buffered formalin was retained for micro-CT analysis. Before scanning, the penis was washed briefly twice in 1× PBS, then put into 70% ethanol and progressively transferred to 100% ethanol. The penis was then incubated in a 10% iodine solution in 100% ethanol for 7 days to provide contrast, then rinsed briefly in 100% ethanol. Micro-CT scanning was performed with a Phoenix Nanotom m (Waygate Technologies GmbH, Wunstorf, Germany) operated using xs control and Phoenix datos|x acquisition software (both Waygate & Inspection Technologies). The echidna penis was mounted in a specimen jar for micro-CT scanning, and X-ray energies and optic geometries were chosen to achieve the best possible contrast and resolution from the scans; this was an X-ray energy of 60 kV, 300 μA, voxel size of 11.5 μm, with a tungsten target and a scan time of 10 min for a total of 1,199 projections. The scan was run in fastscan mode using averaging of 1 and skip of 0 with an integration time of 0.5 s. Given its large size, the echidna penis was scanned in 2 segments using a multi-scan procedure to achieve a higher resolution over the full specimen length. Volume reconstruction was performed using the Phoenix datos|x reconstruction software applying a median filter and ROI filter during reconstruction. The data were exported as 16-bit volume files for analysis. 3D image analysis and segmentation were performed in Avizo v9.7.0 (Thermo Scientific) via the MASSIVE platform [Goscinski et al., 2014].

Magnetic Resonance Imaging

MR images of a single male adult echidna were acquired at the Centre for Magnetic Resonance, Gehrmann Laboratories, The University of Queensland, as described by Johnston et al. [2006a]. Briefly, the echidna was scanned using a Bruker (Ettlingen, Germany) AVANCE spectrometer interfaced to an Oxford 2T whole body magnet. T2-weighted spin echo images were acquired with transmission from the body coil and signal acquisition from a receive-only human head coil.

Visualisation

Histological sections were examined using an Olympus BX51 microscope (Olympus Corporation) and photographed with the attached Olympus DP70 camera (Olympus Corporation). Additional photos were taken using a stereo microscope (SZX9, Olympus Corporation) and the attached Olympus camera (DP25; Olympus Corporation).

Results

External Gross Anatomy

In adult males, the penis is retracted into the preputial sheath, which lies closely apposed to the ventral body wall, just above the opening of the cloaca (Fig. 1). The retracted penis is curved in a reverse "C" shape within the preputial sheath. The head of the penis sits just above a sphincter on the ventral surface of the cloacal wall that it uses to pass into the cloaca so that it can exit through the cloaca during copulation (Fig. 1e). On the ventral side of the penis, a levator muscle runs the length of the first 3 quarters of the penis (Fig. 1g). At the end of the levator muscle, a ventral groove delimits the glans of the penis (Fig. 1c, g). Most of the skin of the penis is relatively smooth with epithelial spines present mainly at the distal tip, surrounding the glans (Fig. 1h). The 4 rosettes of the glans penis are surrounded at their distal end by concentrically arranged circles of penile epithelium around a central shallow depression. Two presumed retractor penis muscles attach at the base of the penis (Fig. 1i).

Internal Gross Anatomy

The echidna penis is highly vascular and contains no baculum. The shaft of the penis consists essentially of a

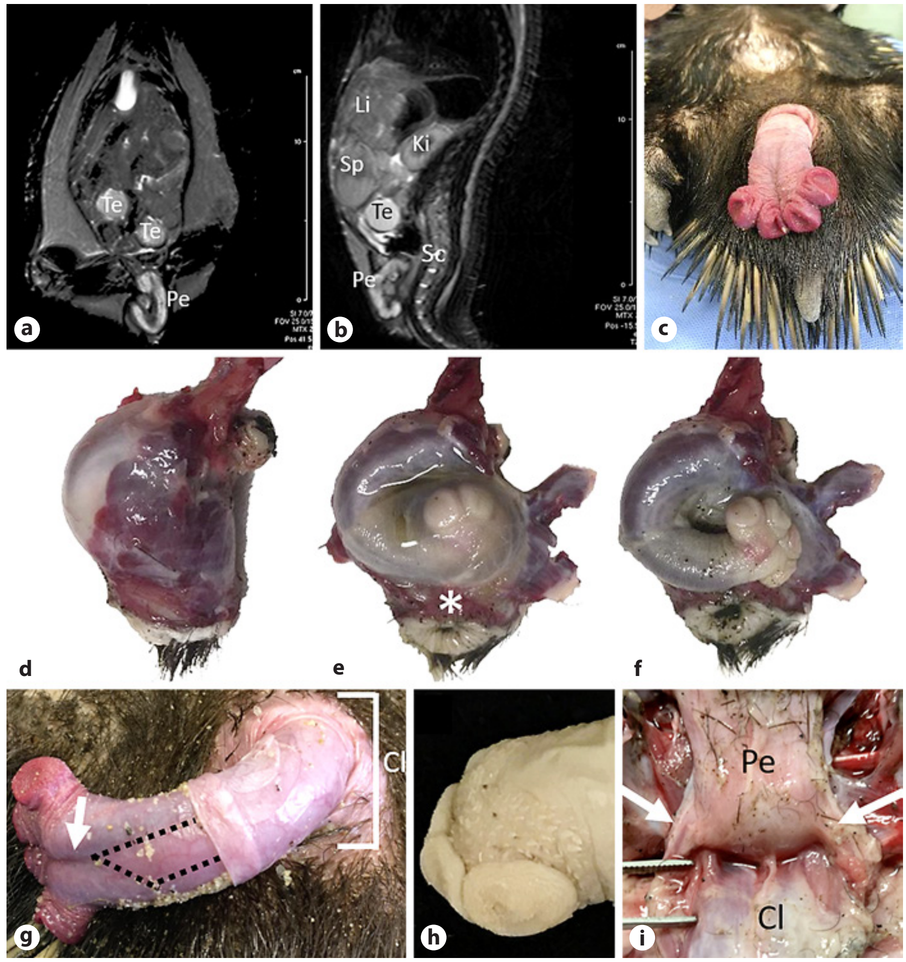

Fig. 1. Location and external anatomy of the echidna penis. **a**, **b** Coronal and sagittal MRI scans showing the location of the penis in the retracted state. **c** Gross morphology of the non-erect penis that has been manually everted from the cloaca of an anesthetised echidna. **d–f** Dissection of the penis in situ showing progressive removal of layers of the ventral body wall (**d**), preputial sheath with cloaca exit (*) indicated (**e**), and exposed penis (**f**). **g** Ventral side of the everted penis showing levator muscle (proximal location indicated by dotted lines), ventral groove (arrow), preputial sheath and location of cloaca. **h** Terminal end of a fixed penis showing the epithelial spines. **i** Dorsal side of penis and ventral side of the cloaca in situ showing presumed penile retractor muscles connected to the base of the penis (arrows). Te, testis; Pe, penis; Li, liver; Sp, spleen; Ki, kidney; Sc, spinal cord; Cl, cloaca.

tube containing the urethra and columns of erectile tissue, the corpus cavernosum, and the corpus spongiosum. Histological examination confirmed the presence of the levator muscle on the ventral side of the penis and its termination just prior to the ventral groove that denotes the beginning of the glans penis (Fig. 2, 3k).

The entire penis is covered by an epithelium and a layer of collagen that encircles the external circumference with the collagen forming the connective tissue between the corpus cavernosum and the 2 sides of the corpus spongiosum (Fig. 3c–f). The corpus cavernosum commences as 2 separated bodies that come together at the base of the penis to unite and form a single mass on the ventral-lateral sides of the urethra (Fig. 3c, d, g, h). The corpus cavernosum contains extensive blood lacunae that are separated by thick collagen septa that extend to the epithelium (Fig. 3l). Termination of the corpus cavernosum occurs slightly distal to the termination of the levator muscle and the first bifurcation of the urethra. In the proximal end of the penis, the corpus spongiosum consists of 2 halves that are separated by a distinct collagenous septum on the dorsal side of the penis (Fig. 3c, g). Each lateral half extends ventrally until they meet the distal sides of the levator muscle that lies on the ventral surface of the corpus cavernosum (Fig. 3c, g). The corpus cavernosum contains thick walls that are dissected by collagen septa that extend to the epithelium (Fig. 3m). Distal to the termination of the levator muscle, the corpus spongiosum extends around the ventral surface of the penis but meets at an additional collagenous septum (Fig. 3d, h). Distal to the termination of the corpus cavernosum, the ventral and dorsal septa join medially (Fig. 3e, i). Once the urethra has reached the glans proper, the corpus spongiosum encircles each urethral bifurcation (Fig. 3f, j, n).

Penile Microanatomy

The urethra is lined by epithelium consisting of a basal layer of columnar epithelia with basally positioned nu-

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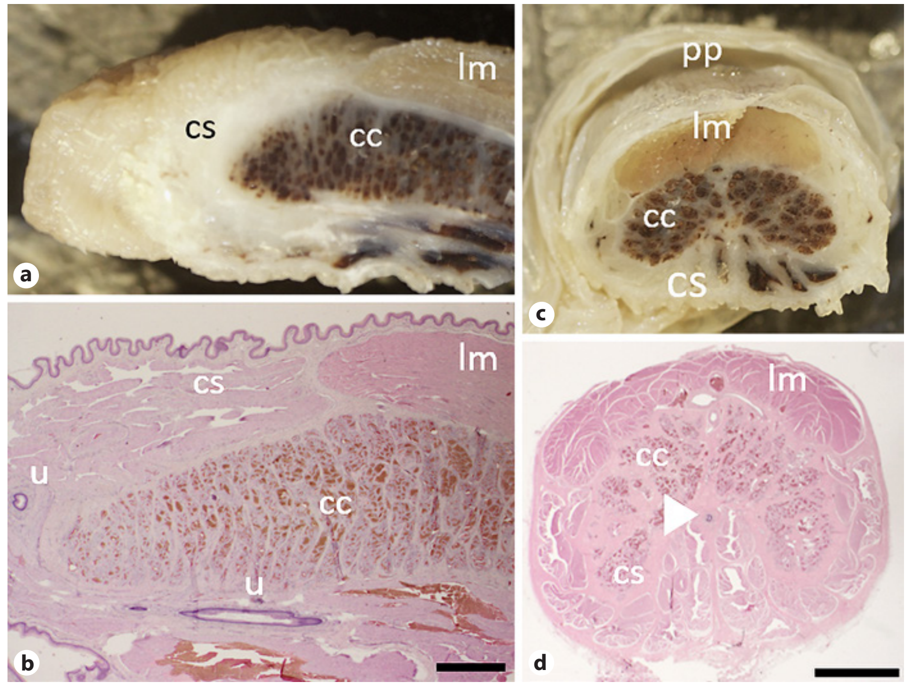

Fig. 2. Gross internal morphology of the echidna penis. **a** Bisection of the front half of a fixed penis in the sagittal plane showing the levator muscle and corpus cavernosum. **b** H&E stained section of the same tissue in the sagittal plane showing location of the levator muscle, corpus cavernosum, corpus spongiosum, and urethra. **c** Dissection of a fixed penis in the transverse plane showing the preputial sheath, levator muscle, corpus cavernosum, and corpus spongiosum. **d** H&E stained section of the same tissue in the transverse plane showing the location of the levator muscle, corpus cavernosum, corpus spongiosum, and urethra (arrow). The preputial sheath has been removed. lm, levator muscle; cc, corpus cavernosum; cs, corpus spongiosum; u, urethra; pp, preputial sheath. Scale bars, 2 mm.

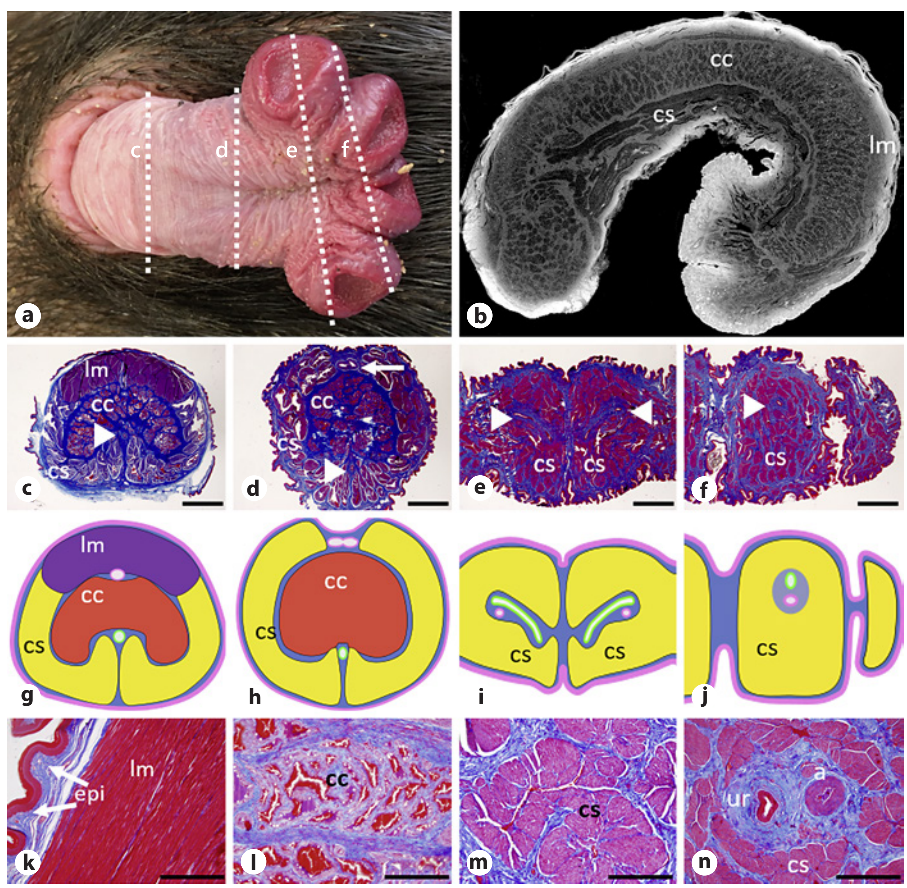

Fig. 3. Structural changes in internal penile anatomy from proximal to distal. **a** Everted penis of an anesthetised adult showing the location of the sections below. **b** Micro-CT sagittal cross section through the middle of the entire penis. **c–f** Mallory-stained transverse sections at different levels. **c** Section at the proximal end of the penis. **d** Section distal to the termination of the levator muscle showing the bifurcation of the penile artery (arrow). **e** Section distal to the termination of the corpus cavernosum, the migration of the urethrae and the collagenous septum (blue) separating the 2 sides of the corpus spongiosum. **f** Section of one half of the distal glans penis after the migration of the urethra at a point where it is surrounded by the corpus spongiosum. Arrowheads indicate the position of the penile urethra. **g–j** Line diagrams highlighting the same structures as in **c–f** with the urethra in green and major artery in pink. **k–n** High magnification of Mallory-stained sections of the major tissues of the penis. cc, corpus cavernosum; cs, corpus spongiosum; lm, levator muscle; epi, epithelium; a, penile artery; ur, penile urethra. Scale bars, 2.5 mm (**c–f**) and 500 μm (**k–n**).

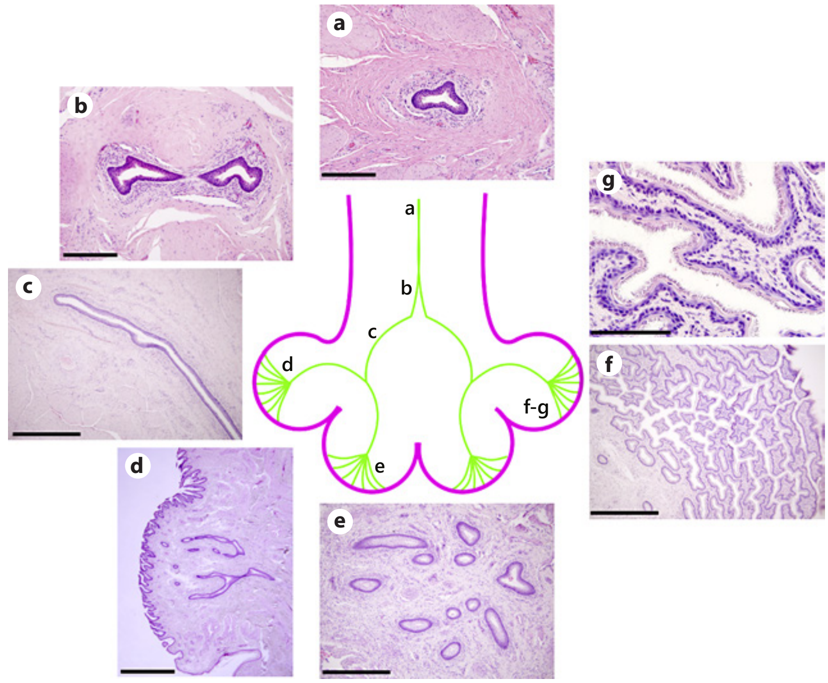

Fig. 4. Characterisation of penile urethra bifurcations. **a** Urethra proximal to the first bifurcation. **b** First bifurcation of the urethra. **c** Migration of the urethra laterally. **d** Initial radiation of urethra to glans. **e** Additional radiations of urethra. **f** Radiation and exit of urethra from glans. **g** Enlarged details of the convoluted glans epithelium. Scale bars, 200 μm (**a**, **b**), 500 μm (**c**, **e**, **f**), 1 mm (**d**), and 100 μm (**g**).

clei and additional layers of squamous stratified epithelia (Fig. 4a–c, e). At the base of the penis, the urethra is located medially, immediately dorsal to the corpus cavernosum, and is positioned in the dorsal septum that separates the 2 sides of the corpus spongiosum (Fig. 2d, 3c, g). As the urethra progresses distally, it extends dorsally until it is located just above the epithelial surface (Fig. 3d, h). The urethra remains as a single tube until the most distal end of the penis shaft when it bifurcates twice in close succession to communicate with each of the 4 rosette glans. The first urethral bifurcation occurs close to the lateral plane of the proximal rosette glans where the exit of the initial glans occurs (Fig. 4b). Soon after, the corpus cavernosum terminates, and the 2 urethrae begin to migrate laterally away from each other (Fig. 3e, i, 4c). The urethrae migration results in each being surrounded by the corpus spongiosum (Fig. 3f, j, n). Close to the termination of the glans, each urethral branch undergoes further divisions and radiates out to connect with the highly convoluted and extensive distal openings of the penile urethra which are lined with keratinised stratified squamous epithelium (Fig. 4d, f, g). No valves or musculature are present around the bifurcations of the urethra (Fig. 4b). Micro-CT analysis confirmed the histological observations of the urethral bifurcations (Fig. 5f).

The thick-walled penile artery lies ventral to the corpus cavernosum, just below the levator muscle (Fig. 3g, 5a). This is the major blood vessel that supplies blood to the penile tissues. It bifurcates soon after the termination of the levator muscle just before the initial bifurcation of the urethra (Fig. 3d, h, 5b). After bifurcation of the vessel, it migrates laterally through the corpus spongiosum and descends to the centre of the glans (Fig. 5c). After the second urethral bifurcation, the migration of each branch of the urethra to the centre of each glans meets the respective branch of the blood vessel so that they are located adjacent to each other (Fig. 3n). By this stage, the 2 distinct sides of the corpus spongiosum separated by a septum completely encircle each urethra (Fig. 3f). Micro-CT analysis confirmed the bisection of the blood vessel and its tracking of the penile urethra (Fig. 5e). Furthermore, micro-CT analysis confirmed that the 2 sides of the corpus spongiosum remain separate for the entire length of the penis, bisected by the penile artery (Fig. 5f).

Connection of the Urogenital Sinus to the Urethra

The vas deferens enters into the urogenital sinus (UGS) just distal to where the ureters enter from the bladder (Fig. 6a). The penile urethra branches off the urogenital sinus just proximal to where the urogenital

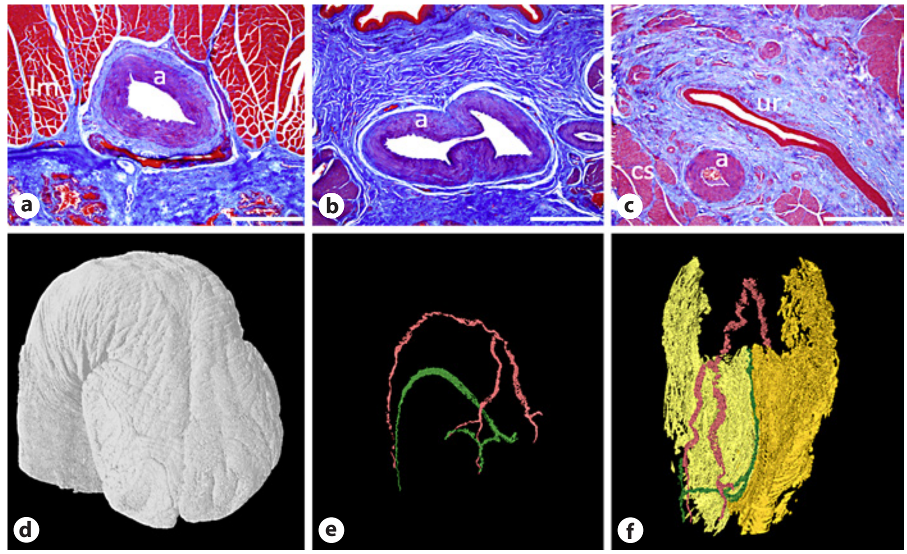

Fig. 5. Bifurcation of the major penile artery in relation to the bifurcation of the urethra and the separation of the corpus spongiosum. **a** Just prior to termination of the levator muscle, the major penile artery is a single vessel. **b** Just distal to the termination of the levator muscle, the artery splits into two. **c** After the split, each branch of the artery migrates dorsolaterally and follows the progress of the respective branch of the penile urethra. **d** 3D micro-CT volume rendering of the front of the penis. **e** 3D micro-CT reconstruction in the same orientation as **d** of the initial bisection of the penile artery (pink) and the urethra (green) and the subsequent bifurcation of the artery and urethra on the anatomical right side. **f** 3D micro-CT reconstruction from the anterior view of the 2 distinct corpora spongiosa in orange and yellow, the urethra separating them and penile artery. Im, levator muscle; a, penile artery; ur, urethra; cs, corpus spongiosum. Scale bars, 500 μm.

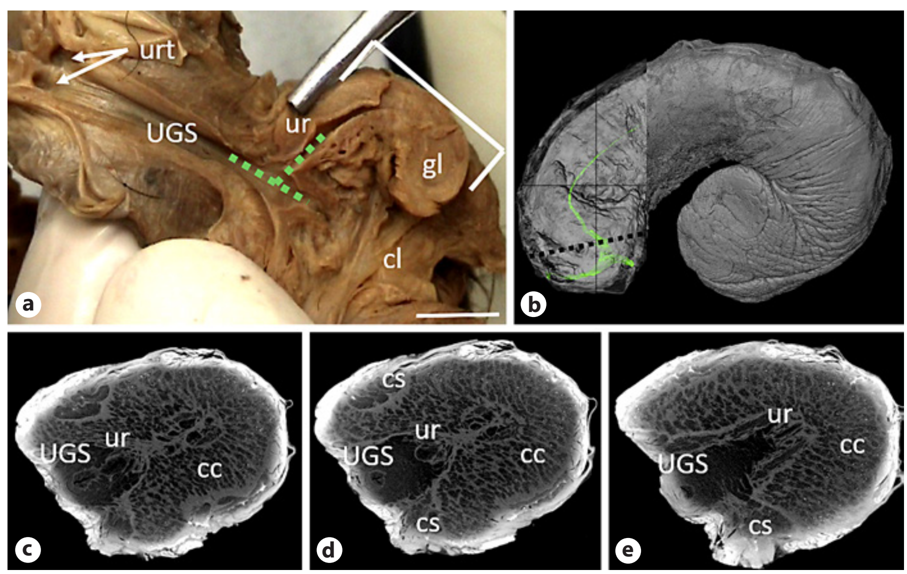

Fig. 6. Connection of the urogenital sinus to the urethra. **a** Formalin-fixed dissected tract from a museum specimen showing connection between the ureters and the UGS and the connection between the UGS and the urethra proximal to the entry of the UGS into the cloaca. **b** 3D micro-CT transparent image of the back half of the penis and UGS with the penile urethra and UGS (green) visible inside showing the connection of the urethra to the UGS. The termination of the base of the phallus is indicated by the dotted line. **c–e** Micro-CT transverse sections of the base of phallus showing the progression of the urethra from the UGS into the middle of the penis and the musculature surrounding the UGS. UGS, urogenital sinus; ur, urethra; urt, ureters; gl, penile glans; cl, cloaca; cc, corpus cavernosum; cs, corpus spongiosum. Scale bar, 1 cm.

sinus enters the cloaca (Fig. 6a). The UGS, proximal to its entry into the cloaca, runs as a continuous tube that is closely apposed to the base of the penis (Fig. 6b–e). The micro-CT analysis established that soon after the urethra branches off from the UGS, it migrates to the middle of the penis (Fig. 6c–e) before progressing towards the glans (Fig. 6b). Micro-CT analysis also highlighted the extensive musculature surrounding the UGS proximal to, and at the point of connection with, the penile urethra (Fig. 6c–e).

Discussion

The copulatory apparatus in mammals has taken 2 separate trajectories during its evolution, the monotreme and the therian model of the marsupials and eutherians. The echidna has a distinctive male sex organ, and this study has defined the unique arrangement of the ducts and supporting tissues in the adult echidna penis.

There are few detailed visual depictions of the structure and functions of the male echidna reproductive tract, especially its most unique features such as the penis and the quadrifurcate urethra [Home, 1802b; Keibel, 1904; Griffiths, 1968; Johnston et al., 2007b]. This study confirmed that the echidna has a musculo-cavernous penis with intersections of collagen to support the major tissues. All bifurcations of the ducts within the adult echidna penis were restricted to the distal quarter of the penis. This disproves an earlier hypothesis, based on external observations of the erect penis, and on the similarity in form and function of the echidna penis with the paired squamate hemipenes [Dowling and Savage, 1960], that the first urethral bifurcation occurred in the proximal part of the penis [Johnston et al., 2007b]. Although the platypus glans penis has only 2 lobes, the urethra bifurcates once in the glans and each urethral branch then undergoes further divisions close to the termination of the glans and radiates out to connect with the distal openings of the glans via foliate papillae, similar to the echidna [Temple-Smith, 1973]. In the flaccid echidna penis, the urethra in the lateral glans curves backward relative to the penile shaft. As the glans becomes tumescent, it rotates from lateral to more medial due to the unilateral erection which moves the contained urethral branches to be more in line with the penile shaft.

Previous reports suggested that multiple aspects of the form and function of the echidna penis and squamate hemipenes are homologous [Owen, 1868; Boas, 1891; Gerhardt, 1909; Johnston et al., 2007b]. One homologous feature are the apparent alternating constrictions of left and right urethral openings in the echidna, and the alternating use of left and right hemipenes in squamates [Dowling and Savage, 1960; Johnston et al., 2007b]. However, the distal location of all the urethral bifurcations in the echidna and complete separation of the left and right sides of the reproductive system in squamates that empty independently into their respective hemipenes are not supportive of a homologous structure. This study instead demonstrates that the echidna penis bears more similarity to phalluses of crocodilian and chelonid reptiles. In both the crocodilians and turtles, sperm enter into the urodaeum and then are directed into a single spermatic sulcus at the start of the phallus [Zug, 1966; Johnston et al., 2014; Gredler et al., 2015; Larkins and Cohn, 2015]. In addition, turtles have elaborate and diverse external genital anatomy, and in many species the sulcus bifurcates in the glans, similar to the monotremes. For example, the genus *Trionyx* has a 5-lobed glans and the sulcus bifurcates at the entrance to the glans and again at the base of the lobes resulting in 5 exits, but it is unknown whether all glans are used simultaneously [Zug, 1966]. Therefore, this study provides further evidence for a common ancestral origin of the amniote phallus.

The mechanisms behind the control of the urethral bifurcations and the likely alternation between the 2 sides of the echidna penis have previously been elusive [Johnston et al., 2007b]. One prediction was that it would involve a valve mechanism to control the flow of semen at the initial bifurcations. However, we found no evidence for any valve or muscular control at the initial split of the urethra. Another possible explanation was that it could depend on bifurcation of the vascular tissue. In marsupials with a bifid penis, the corpus cavernosum commences as 2 separate bodies that merge at the base of the penis and then bifurcate distally in the glans [van den Broek, 1910; Woolley and Webb, 1977]. However, in the echidna, the corpus cavernosum commences as separate bodies that merge at the base of the penis and remain as a singular structure until its termination.

The corpus spongiosum in the echidna also commences as 2 separate bulbs, but these 2 bulbs never unite and remain separated by a collagenous septum. This is in contrast to marsupials where although proximally the corpus spongiosum has 2 separate lobes, distally, they unite medially to surround the urethra and bifurcate only in the glans of those species that have a bifid penis [Owen, 1868; Woolley and Webb, 1977]. In other mammals, blood flow to the corpus spongiosum ensures the urethra remains open during erection. Furthermore, each side of the corpus spongiosum in the echidna only surrounds the urethra distal to its initial bifurcation and the termination of the corpus cavernosum. Proximal to this, the urethra lies between the corpus cavernosum on its ventral-lateral side and the corpus spongiosum on its dorsal-lateral side. In addition, the major penile artery bifurcates soon after the termination of the levator muscle, close to the initial bifurcation of the urethra. Distal to each arterial bifurcation, each artery then passes dorsolaterally to the centre of each side of the penis and subsequently closely follows the path of each urethra. These 2 observations combined suggest that the echidna forms a double glans penis and that unilateral control of blood flow to only one side of the corpus spongiosum enables its urethra to be kept open, and this could provide a mechanism by which unilateral ejaculation of the glans penis is controlled.

Our results confirm that the semen enters the penile urethra through an offshoot of the UGS, which at the point of connection is surrounded by extensive muscular layers. Of the 3 alternate mechanisms proposed to direct the semen into the urethra and not the cloaca, from our anatomical observations we conclude that enlargement of the penis at erection is the most likely option to facilitate this. This study provides the first detailed anatomical study of the echidna penis and provides a mechanism by which only 2 of the 4 glans penile rosettes could become erect at any one time and result in unilateral ejaculation. Our analysis of the echidna penis suggests that modifications of an ancestral (reptilian) programme of urogenital development could have generated the curious morphological structures found in the external genitalia of monotreme mammals.

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Statement of Ethics

The University of Queensland Animal Experimentation Ethics Committees approved all sampling for echidnas, in accordance with the Guidelines of the National Health and Medical Research Council of Australia, 2013.

Conflict of Interest Statement

The authors declare no conflict of interests.

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Author Contributions

J.C.F., S.D.J., and M.B.R. designed research; J.C.F., C.M., and S.D.J. performed research; J.C.F., S.D.J., M.B.R., and M.P. collected tissues; J.C.F., C.M., S.D.J., A.R.E., G.S., and M.B.R. analysed data; and J.C.F, S.D.J., G.S., and M.B.R. wrote the paper. All authors read and approved the final version of the manuscript.

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