Differentiation 116 (2020) 26–37

Contents lists available at ScienceDirect

Differentiation

journal homepage: www.elsevier.com/locate/diff

Anatomy of the mouse penis and internal prepuce

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ARTICLE INFO

Keywords:
Glans penis
External prepuce
Internal prepuce
Mouse

ABSTRACT

This paper addresses a confusing issue of preputial anatomy of the mouse. The term "internal prepuce" was used in 2013 to describe a preputial structure integral to the mouse glans penis. Subsequently in 2015 the same term was applied by another group to describe entirely different morphology, generating confusion in the literature. Because it is inappropriate to use the same term to describe entirely different structures, we take this opportunity to provide further descriptive information on the internal prepuce of the mouse employing gross dissection, analysis of serial histologic section sets, three-dimensional reconstruction, scanning electron microscopy and immunohistochemistry. For this purpose, we review and illustrate the relevant literature and provide some additional new data using standard morphological techniques including immunohistochemistry. The mouse internal prepuce is integral to the glans penis and clearly is involved in sexual function in so far as it contains a major erectile body innervated by penile nerves. The development of the mouse internal prepuce is described for the first time and related to the development of the corpus cavernosum glandis.

1. Introduction

This paper is a review of mouse penile anatomy and mostly consists of previously published data augmented with new observations. The new data are derived from gross dissections and standard hematoxylin and eosin stained histologic sections.

2. Review and results

Human hypospadias is an abnormality of penile urethral development and is characterized by (a) an ectopic urethral meatus on the ventral aspect of the penis, (b) an abnormal urethral spongiosum, (c) penile curvature and (d) foreskin abnormalities (Baskin, 2000, 2017). This constellation of human anomalies is defined solely by anatomy. For many years mice have been utilized as a model for human hypospadias, and accordingly the effects of teratogenic agents (exogenous estrogens, anti-androgens or targeted genetic mutations) have been used to evaluate whether the resultant malformations provide mouse models relevant to human hypospadias, even though mouse penile anatomy differs substantially from human penile anatomy (Cunha et al., 2019). In 2008 we became concerned regarding diagnostic features that we and others had previously used to assess "mouse hypospadias". After critical review of the literature, we concluded that the extant literature on normal adult mouse penile morphology required a more precise and meaningful anatomic vocabulary as a basis for interpreting experimental "mouse hypospadias". As a starting point we accepted the previously established definition and distinction between the mouse (and rat) glans versus body of the penis (Goyal et al., 2007; Heath et al., 2011; Purkart et al., 2020), which has been verified by gross dissection (Rodriguez et al., 2011), by MRI (Phillips et al., 2015) and by histologic sections (Fig. 1A). This terminology distinguishing glans versus body of the mouse penis is accepted by GUDMAP (Figs. 1B and 4B & D). The mouse glans is an outward projection from the body surface lying within the external preputial space (Figs. 1 and 4C). The mucosal lining of the external prepuce reflects onto the surface of the glans (to become penile epidermis) in the depth of the preputial space (large black arrowheads in Figs. 1A & 4C). In contrast, the body of the mouse penis lies deep to the body surface, and thus in the resting state is not associated with an epithelial layer (Figs. 1 and 4).

Critical examination of adult mouse penile anatomy over the course of our studies led to some new anatomical terms to augment previous anatomic descriptors. Taken together this standardized terminology has provided the vocabulary for research on normal and abnormal penile development and anatomy of the mouse penis and is now established within the lexicon of GUDMAP (Fig. 1B). The distal cartilaginous projection of the adult mouse glans penis has been named the male urogenital mating protuberance (MUMP) (Rodriguez et al., 2011; Yang et al., 2010), a term widely used (Figs. 1–4). Proximally the MUMP fuses

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https://doi.org/10.1016/j.diff.2020.09.002
Received 8 August 2020; Received in revised form 18 September 2020; Accepted 21 September 2020
Available online 30 September 2020

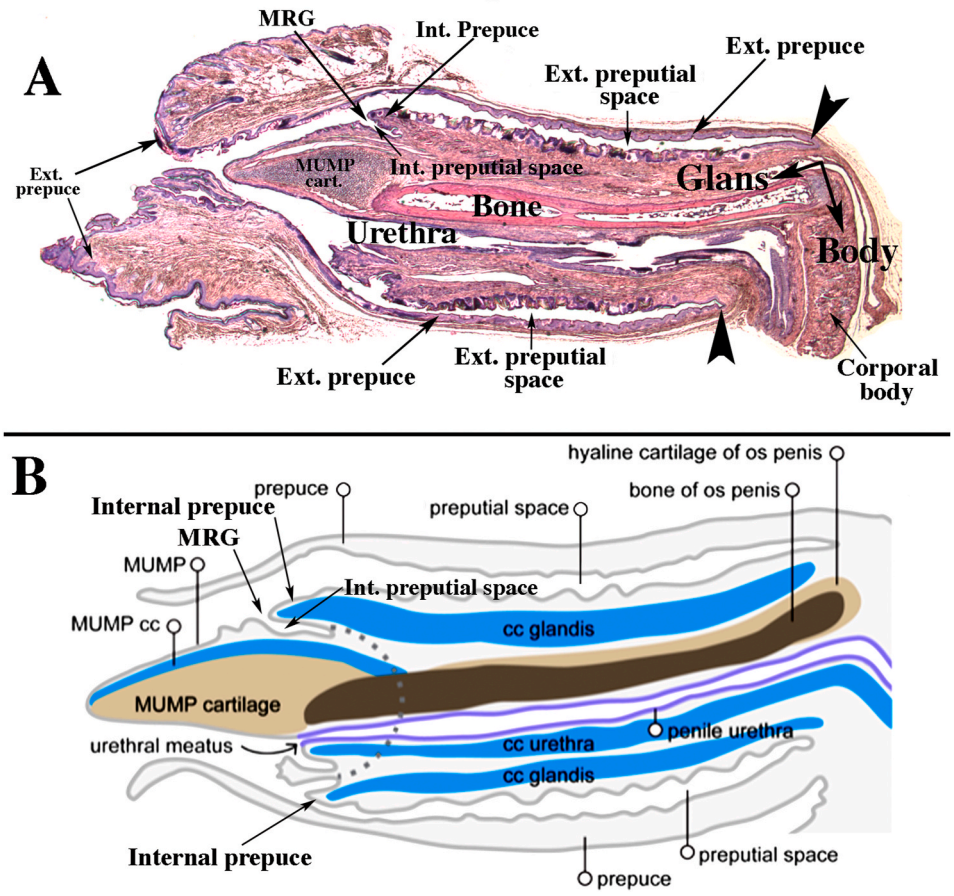

Fig. 1. (A) A parasagittal section of an adult mouse penis within the external preputial space. Note the junction of the glans and body of the penis at the right angle bend. The glans resides within the external preputial space. In this parasagittal section the internal prepuce is seen dorsal to the MUMP cartilage. The internal prepuce is defined distally by the MUMP ridge groove (MRG). The depth of the internal preputial space varies along its circumference and is shallow in the mid-dorsal position. The mucosa of the external prepuce reflects onto the surface of the penis (large black arrowheads) to become penile epidermis. (B) Figure adapted from GUDMAP of the adult mouse glans penis. Note the common morphology and terminology between section A and the GUDMAP drawing. The space, within which the glans resides, is one continuous preputial space (now called the external preputial space in [A]). Not originally labelled in (B) is the internal prepuce even though it is accurately drawn. The dotted line in (B) gives a reasonable indication of the internal preputial space whose depth varies circumferentially. (Adapted from GUDMAP).

with a ridge on the surface of the glans, which is appropriately named the MUMP ridge (Rodriguez et al., 2011) (Figs. 2–5). The MUMP ridge forms via fusion of multiple bilateral elements (Blaschko et al., 2013; Liu et al., 2018) (Fig. 3). The MUMP ridge is partially divided by a ventral cleft (Figs. 2A, 3 and 5A), which suggested that the MUMP ridge formed via incomplete fusion of bilateral halves (Blaschko et al., 2013; Liu et al., 2018). This cleft is appropriately called the MUMP ridge cleft (also simply called the ventral cleft) (Rodriguez et al., 2011; Yang et al., 2010). The MUMP ridge is separated proximally from the rest of the glans by a groove, called the MUMP ridge groove, which is the opening of the internal preputial space as will be explained below (Rodriguez et al., 2011) (Figs. 1–5).

Descriptions and terminology for erectile bodies within the adult mouse penis were proposed many years ago by Murakami, who added the following anatomical terms to the penile anatomical vocabulary: (a) the bilateral corpora cavernosa and their fusion to form the corporal body located within the body of the penis (Fig. 1A); (b) the bilateral corpora cavernosa urethrae that originate distally in the urethral flaps (Fig. 5b); and (c) the corpus cavernosum glandis (Murakami, 1987) (Fig. 8). The corpora cavernosa urethrae extend distally into projections into the urethral lumen call urethral flaps (Fig. 8A) (Mahawong et al., 2014a; Rodriguez et al., 2011). The MUMP corpora cavernosa were subsequently discovered and described in the glans as bilateral erectile bodies in close association with the MUMP cartilage (Rodriguez et al., 2011) (Figs. 3 & 8A-B).

Further analysis lead to the discovery that the mouse has two prepuces, an external prepuce and an internal prepuce that is integral to the mouse penis (Blaschko et al., 2013) (Fig. 7B). Perhaps before discussing the two mouse prepuces, the term prepuce should be defined. Standard dictionaries define the prepuce as "the fold of skin that covers the head of the penis". Cold and Taylor (1999), in their monograph entitled "The Prepuce", indicate that "the prepuce is an integral, normal part of the external genitalia that forms the anatomical covering of the glans penis".

The mouse glans penis lies within a single continuous preputial space as described in GUDMAP (see https://www.gudmap.org/tutorials/urogenital-dev/devmrs.html) and verified/illustrated by numerous investigators (Cunha et al., 2015; Hennefarth et al., 2020; Mahawong et al., 2014a, b; O'Neill et al., 2017; Rodriguez et al., 2011; Sinclair et al., 2016c) (Figs. 1 and 4). However, in 2013 the mouse internal prepuce and internal preputial space were discovered (Blaschko et al., 2013). This observation necessitated a change in terminology. The single unitary "prepuce and preputial space" described previously (Rodriguez et al., 2011) is now designated as external prepuce and external preputial space (Fig. 1A and B, 4, 5E-F) in distinction to the mouse internal prepuce and internal preputial space (Figs. 1, 5 and 8). The internal prepuce as described by Blaschko et al. conforms precisely with the definitions of prepuce as stated above (Cold and Taylor, 1999).

The term external prepuce connotes two anatomical entities: (a) the prominent hair-bearing perineal appendage (Fig. 4A) and (b) the unitary mucosal lining of the external preputial space which begins at the preputial meatus on the perineal appendage (external prepuce) and terminates proximally in the depth of the external preputial space by reflecting onto the surface of the glans (Figs. 1A, 4B–C).

In the embryo, the external prepuce is formed via fusion of the preputial shelves with the genital tubercle (GT) (Liu et al., 2018; Perriton et al., 2002; Petiot et al., 2005; Suzuki et al., 2002). After the bilateral embryonic preputial shelves fuse in the ventral midline, they grow distally to completely cover the GT and in so doing the preputial lamina is laid down (Liu et al., 2018). Throughout the current literature of mouse penile development the term preputial lamina has been used both in the embryo and in the neonate, but to be more specific the "preputial lamina" designated in the past should now be called the

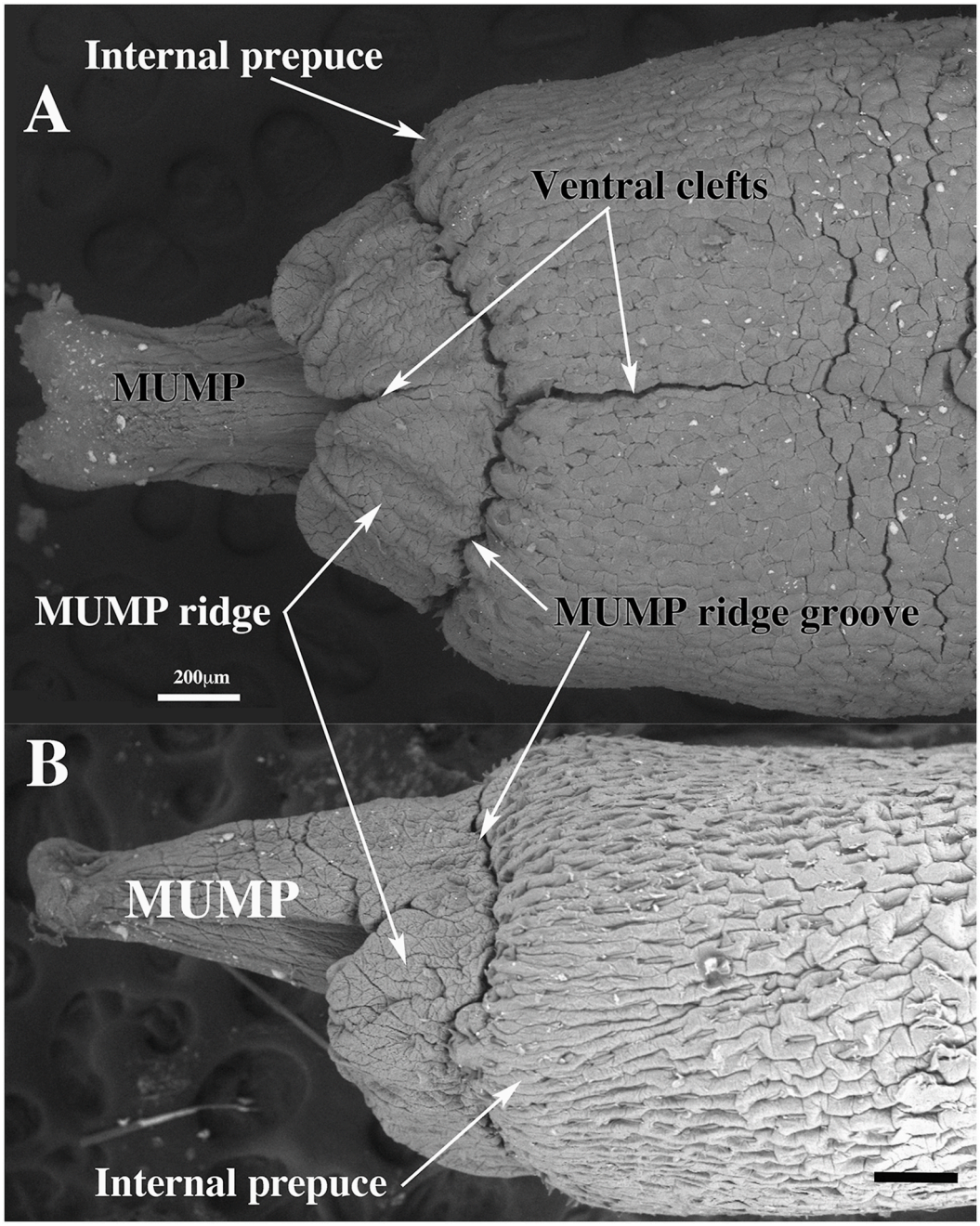

Fig. 2. Scanning electron micrographs (SEMs) of the adult mouse penis (external prepuce removed) in ventral view (A) and side view (B). Note that the MUMP is fused with the MUMP ridge (B) and extends distally beyond the MUMP ridge (A & B). The MUMP ridge groove demarcates the distal aspect of the internal prepuce space (Fig. 1A). Both the MUMP ridge and the internal prepuce have ventral clefts (A). (Adapted from Blaschko et al., 2013 with permission).

external preputial lamina to distinguish it from the internal preputial lamina to be described below (Fig. 6).

In late fetal life and for the first 3–4 weeks postnatal the solid external preputial lamina separates the stromal wall of the external prepuce from tissues/structures of the glans penis (Fig. 6C). In the 4th week postnatal the external preputial lamina delaminates (Cripps et al., 2019; Mahawong et al., 2014b) to create the external preputial space (Figs. 1, 4C and 5B, E-F). The internal preputial lamina can be recognized at 5 days postpartum as shallow epithelial ingrowths penetrating proximally into the developing glans (Fig. 6A). These epithelial ingrowths are more prominent at 10 days postnatal and penetrate more proximally (Fig. 6B). A characteristic feature of the developing internal prepuce is that mesenchymal condensations representing the rudimentary corpus cavernosum glandis (CCG) arise distally within the developing internal prepuce. This is particularly evident at 10 days postpartum in both parasagittal and transverse sections (Fig. 6B and C), in the adult mouse glans (Fig. 8) and is corroborated in Fig. 3 of Hennefarth et al. (2020). As stated above, before the discovery of the mouse internal prepuce, the more general terms (prepuce and preputial space) were adequate. However, with the discovery of the mouse internal prepuce, more precise terminology is required: external prepuce, external preputial space, internal prepuce, and internal preputial space.

When first reported, the mouse internal prepuce was suggested to be homologous to the human prepuce based upon the fact that the mouse internal prepuce and the human prepuce are both integral to the glans penis (Fig. 7), and thus both conform with the definition of prepuce (Cold and Taylor, 1999). The human prepuce is richly innervated by somatosensory and parasympathetic nerves. Sensory nerves terminate in

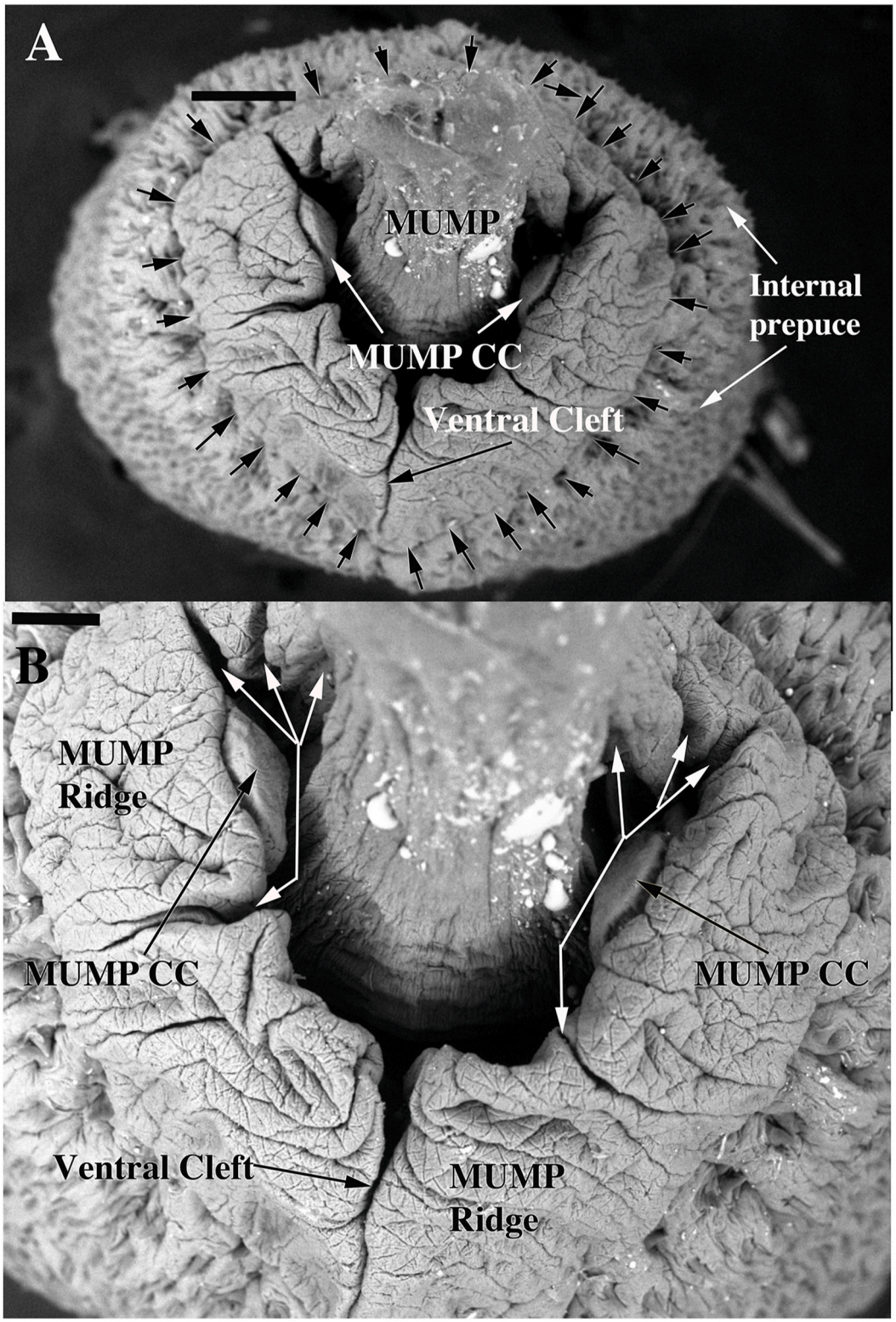

Fig. 3. SEMs of the adult mouse penis (external prepuce removed), end-on view. The urethral meatus is a Y-shaped channel with the ventral cleft being the stem of the Y. Note the minor clefts in the MUMP ridge (white arrows in [B]) as well as the ventral cleft that suggests that the MUMP ridge formed via fusion of individual subunits. In (A) the MUMP ridge groove is denoted by multiple small arrows. MUMPCC = MUMP corpora cavernosa. (Adapted from Blaschko et al., 2013 with permission).

a variety of encapsulated receptors which are known to play an important role for sexual function (Cold and McGrath, 1999; Cold and Taylor, 1999).

A major anatomic signature of the internal prepuce is that it contains a major erectile body of the mouse penis, namely the corpus cavernosum glandis, which arises distally within the mouse internal prepuce (Figs. 6, 8A-D). The association of the corpus cavernosum glandis with the internal prepuce can be traced developmentally back to at least 5 days postpartum when the mesenchymal condensation heralding the formation of the corpus cavernosum glandis can be seen (Fig. 6A). At 10 day postpartum, differentiated corpus cavernosum glandis is particularly evident (Fig. 6B and C) and clearly comparable to that seen in adulthood (Fig. 8) (Hennefarth et al., 2020). The presence of this major erectile body within the mouse internal prepuce is consistent with its role in sexual function as is the case for the human prepuce (Cold and McGrath, 1999; Cold and Taylor, 1999). This interpretation is supported by the density of S100-positive nerve fibers, which in transverse sections are indicated as small brown punctate spots surrounded by circles within the internal prepuce (double-headed arrow in Fig. 8E).

After the report of the internal prepuce by Blaschko et al. (2013), a divergent view of the external and internal prepuce was published by Phillips et al. (2015) in their paper entitled "A Comprehensive Atlas of the Adult Mouse Penis". We agree with Phillips' assertion that the "anatomy of the mouse penis is essential for defining the relevance of murine studies to human penis and urethral development" (Phillips et al., 2015). Fig. 1 from Phillips et al. (2015), is an interesting view of adult male mouse external genitalia that needs further exploration. The photograph was obtained by "applying gentle pressure on the abdomen" to extrude the adult mouse penis outward beyond the meatus in the external prepuce (also known as the perineal appendage, see Fig. 4A, this paper). Photographs were taken sequentially to document the process of penile extrusion (Phillips et al., 2015). The external prepuce, MUMP, glans and MUMP ridge are evident in Phillips Fig. 1. The unusual aspect of this figure is the ridge purported to separate the "glans" from the "internal prepuce". This ridge is located at about the midpoint of along the extruded penis. Fig. 9 (this report) is an exact tracing of Fig. 1A from Phillips et al. (2015) with the internal prepuce as described by Blaschko et al. (2013) precisely superimposed and indicated by dashed lines. The average maximal depth of Blaschko's internal prepuce is indicated (Fig. 9) with a red bar representing 616 μm (maximal depth of the internal prepuce). The depth of the internal space varies circumferentially and is shallow in mid-sagittal sections (Figs. 1A & 4C). Clearly the internal prepuce described in 2013 (Blaschko et al., 2013) does not comport with the internal prepuce described in 2015 (Phillips et al., 2015). Using the 616 μm bar as a reference measure, the application of "gentle pressure" by Phillips has extruded the mouse penis 4.3 mm beyond the external prepuce, which appears excessive given the biomechanical parameters involved in mouse erection described by Hennefarth et al. (2020) and illustrated in their paper (see Fig. 6 in Hennefarth et al.), raising questions as to the nature and meaning of the regions of the penis labelled IP in Phillips, Fig. 1A. Another problem with Fig. 1A of Phillips is the position of the Phillips label MR (black MR label) denoting MUMP ridge. Based upon SEM images in Figs. 2, 5 and 7, we have corrected the Phillips terminology as indicated by red labels (Fig. 9). The arrowhead associated with the Phillips MR label is actually the MUMP ridge groove (Figs. 2 and 5A).

The internal prepuce described by Blaschko et al. (2013) begins distally at the MUMP ridge groove and extends to an average depth of 616 μm microns and is demarcated by the lines in Fig. 4D and dashed lines in Fig. 9. This clear inconsistency in terminology between Phillips et al and our study requires examination. Significantly, the ridge separating glans and internal prepuce (G and IP) in Fig. 1 of Phillips et al. is not seen in their mid-sagittal plane MRI slice (Phillips et al. Figs. 4B and 6B), while an unlabeled internal preputial space as we have described is seen in Phillips et al. Fig. 4Eii (compare with Fig. 8, this paper). Phillips Fig. 6B provides some rationale for their labelling of internal prepuce

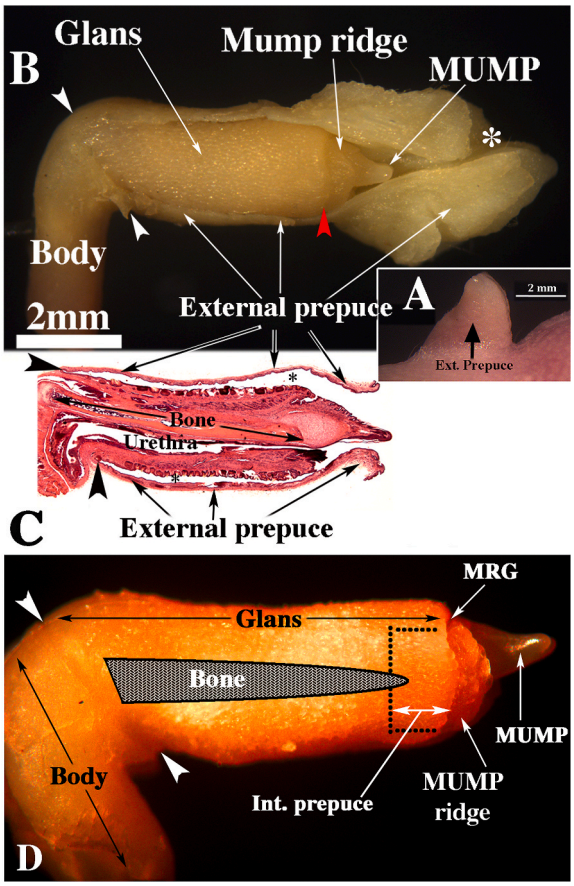

Fig. 4. Illustrations distinguishing the external prepuce from the internal prepuce. (A) Lateral view of the adult nude mouse external prepuce (perineal appendage). An athymic nude mouse was used to see morphology unobscured by hair. (B) Dissection of the adult mouse penis in situ within the external preputial space demarcated by the mucosa of the external prepuce. The white asterisk denotes the preputial meatus near the tip of the external prepuce (perineal appendage), the mucosal lining of which extends without interruption to the junction of the glans with the body of the penis (apposed white arrowheads) where the external preputial mucosa reflects onto the surface of the glans penis. Note also the MUMP, MUMP ridge and MUMP ridge groove (red arrowhead). (C) Mid-sagittal section of the adult mouse penis within the mucosal lining of the external prepuce with the perineal appendage removed. Note the external preputial space (*), the bone, urethra and the reflection of the mucosa of the external preputial onto the surface of the glans (large apposed black arrowheads). (D) Photograph of the adult mouse penis with the position of the os penis superimposed. Note the MUMP, MUMP ridge and MUMP ridge groove (MRG) which defines the distal aspect of the internal prepuce. The depth of the internal preputial space is denoted by the dashed lines (see Fig. 5 for further details). The position of distal aspect of the bone and the depth of the internal prepuce (dotted lines) is accurately displayed based upon analysis of compete serial section sets. (Adapted from Cunha et al., 2015 with permission). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(IP) seen in Phillips et al. Fig. 1A. Fig. 6B (Phillips et al., 2015), is a mid-sagittal plane MRI slice of the adult mouse penis in situ and shows that the perineal appendage is labelled external prepuce (EP), while the mucosal lining of the preputial space is labelled internal prepuce (IP). Referring to Figs. 1 and 4 (this report), it is evident that the lining of the external prepuce (perineal appendage) begins with the lining of the external prepuce at the preputial meatus within the perineal appendage and continues un-interrupted as a singular unitary lining of the preputial space proximally to its termination where it reflects onto the surface of the penis. In so a singular preputial space is defined, which we now term the external preputial space to distinguish form the internal preputial

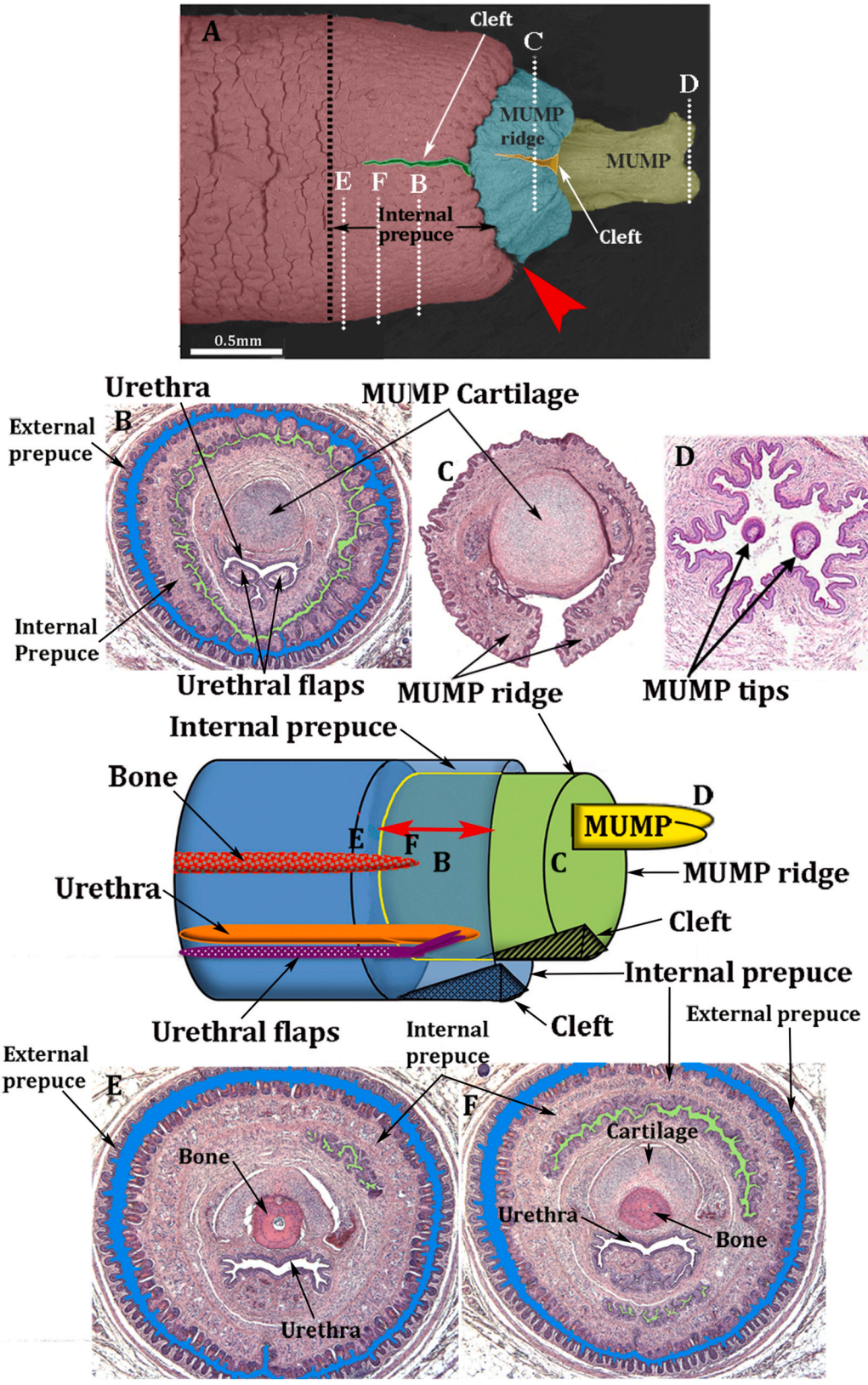

Fig. 5. Diagrammatic three-dimensional reconstruction of the adult mouse penis and an associated SEM, both without the external prepuce (A) as well as transverse sections (B–F) taken at the levels specified in (A) and in the three-dimensional reconstruction (note position of letters B–F). (A) Maximal depth of the internal prepuce is accurately represented with the black dotted line. In sections B, E & F the external preputial space is colored blue, and the internal preputial space is colored green. The stromal wall of the external prepuce (not labelled) is peripheral to the mucosa of the external prepuce (labelled external prepuce). Note in (B) that the internal preputial space (green) completely circumscribes internal penile structures, but that in more proximal sections (E–F) the internal preputial space is only partially present (F) and in (E) has almost completely disappeared. (Adapted from Sinclair et al., 2016a with permission). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

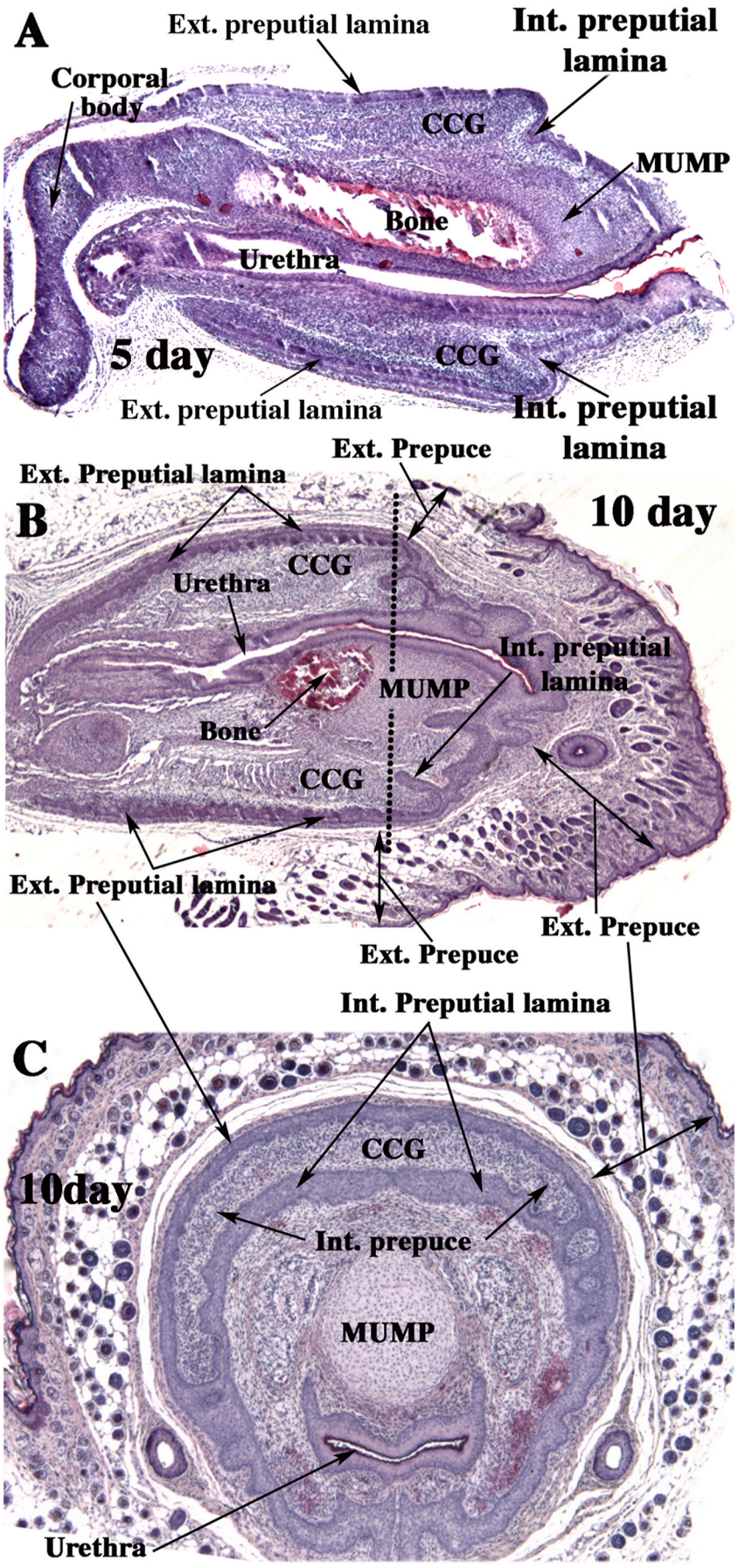

Fig. 6. (A) Mid-sagittal section of the mouse glans penis at 5 days postpartum. Note the shallow epithelial ingrowths which are the rudimentary internal preputial laminae. (B) Para-sagittal section of a penis of a 10-day-old mouse. Note the MUMP, shattered bone, urethra, external preputial lamina, and the external prepuce, which distally contains numerous hair follicles. The internal preputial lamina is also evident. (C) Transverse section of a 10-day mouse penis sectioned as indicated by the dotted line in (B). Note the internal preputial lamina, external preputial lamina and the hair-bearing external prepuce. In all 3 sections note the mesenchymal condensations representing the rudimentary corpus cavernosum glandis (CCG).

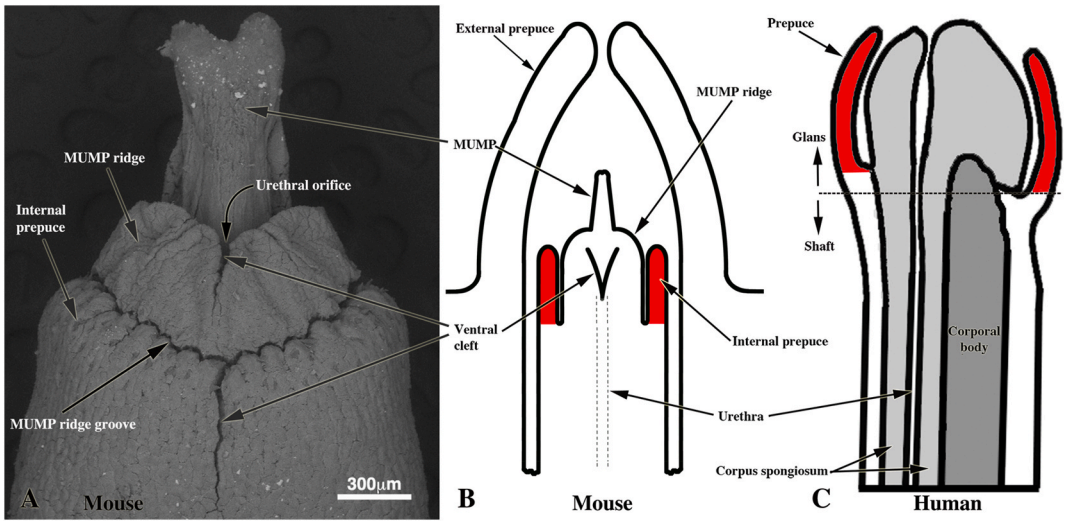

Fig. 7. Homology of the mouse internal prepuce and the human prepuce. (A) SEM of the adult mouse penis. (B) The mouse penile glans lies within an extensive preputial space beginning at the opening of the preputial space distally in the hair-bearing external prepuce (perineal appendage labelled external prepuce in [B]) and ending proximally near the glans-body junction. Drawings of mouse (B) and human (C) penile morphology. Both the mouse internal prepuce and the human prepuce (both red) are integral to the distal penis and encircle the glans. From Blaschko et al., 2013 with permission. (Adapted from Blaschko et al., 2013 with permission). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

space (Figs. 5 and 8). This is the convention used by GUDMAP and is fully supported by mid-sagittal sections of the adult mouse penis in situ (Figs. 1A, 4B–C). Is there justification of giving two different names (Phillips internal prepuce and external prepuce) for one unitary continuous lining mucosa? The term "internal prepuce" was used in 2013 (Blaschko et al., 2013) to describe a structure integral to the mouse glans penis. Use of the same term to describe an entirely different structure is not justified and serves to confuse the literature.

3. Discussion

The goal of this paper is to clarify the anatomy of the mouse penis so that a common universal terminology is well justified and strongly supported with data. The need for re-visiting the issue of anatomic terminology of the mouse penis concerns the prepuce. In 2013 we demonstrated that the mouse has two prepuces, and external prepuce and an internal prepuce (Blaschko et al., 2013). The external prepuce is the perineal appendage and its mucosal sleeve that defines the space (external preputial space) that houses the glans penis. The external prepuce and its associated space are a features common to small rodent-like animals built close to the ground. This anatomical structure is clearly protective for the penis and lacks morphologic homology with the human prepuce as it is not integral to the penis. The internal prepuce of the mouse is homologous to the human prepuce anatomically, developmentally and functionally is so far as (a) both are integral to the glans penis, (b) both are innervated by penile nerves and (c) both develop via formation of preputial laminae (Fig. 6) (Cunha et al., 2020). These shared features emphasize anatomic homology and a role in sexual function, further supported by the presence of the distal aspect of the richly innervated corpus cavernosum glandis within the mouse internal prepuce.

Phillips et al., in 2015 provided observations regarding the mouse external and internal prepuces that are radically different from our views. To resolve an obvious major difference in terminology, we now provide a more detailed description of mouse penile anatomy supported by additional data and critically review the Phillips paper.

Both groups agree on the terminology of the perineal appendage, called the external prepuce. The perineal appendage (external prepuce) has a meatus through which the penis extends distally during erection (Hennefarth et al., 2020). The outer surface of the perineal appendage (external prepuce) is covered by a hair-bearing epidermis. Extending inward (proximally) from the preputial meatus in the external prepuce is a smooth surfaced mucosa (originally also called the prepuce) which lines a space housing the penis (the preputial space). These facts are well known, have been accepted by those in the field, are the lexicon of mouse penile anatomy as illustrated in GUDMAP (Fig. 1B) and are seen most recently in an elegant paper by Hennefarth et al. (2020). Phillips et al., in 2015 renamed the mucosal lining of the preputial space the "internal prepuce", not referring our previous description of an internal prepuce published 2 years earlier in 2013 (Blaschko et al., 2013). A justification for giving different names for one continuous mucosa was not presented, and is a source of confusion given that the term "internal prepuce" had been previously used and clearly described anatomy completely different from the "internal prepuce" proposed by Phillips et al.

Beyond the confusion concerning the use of the term "internal prepuce" for two completely different anatomic entities, the Phillips paper contains a curious inconsistency. Their Fig. 1A of an extruded mouse penis has a zone labelled IP (internal prepuce) and (a) is curiously absent in their MRI wholemount (their Fig. 4A, B & 6B) and (b) is also absent in their 3D reconstruction (their Fig. 5). Their "sagittal" section of the mouse penis does not comport with sagittal sections of the mouse penis reported previously in the literature (compare Phillips Fig. 5 vwith our Figs. 1A and 4C and with the GUDMAP drawing, Fig. 1B). The tip of the arrow labelled MR (MUMP ridge) in Phillips Fig. 1 does not comport with SEMs of the mouse penis (compare Phillips Fig. 1 with SEMs, Figs. 2 and 5A). The Phillips MUMP ridge (labelled MR) is incorrect as it actually indicates the MUMP ridge groove (See red MRG label in Fig. 9). Finally, the extrusion of the mouse penis via application of "gentle pressure to the abdomen" 4.3 mm beyond the hair-bearing external prepuce appears to be excessive based upon biomechanical parameters recently described (Hennefarth et al., 2020), and may be a source of artifactual data in Phillips Fig. 1A.

To provide a broader context for the mouse internal prepuce as an integral component of the glans penis, evidence shows that internal prepuce arises from an ingrowth of the internal preputial lamina that

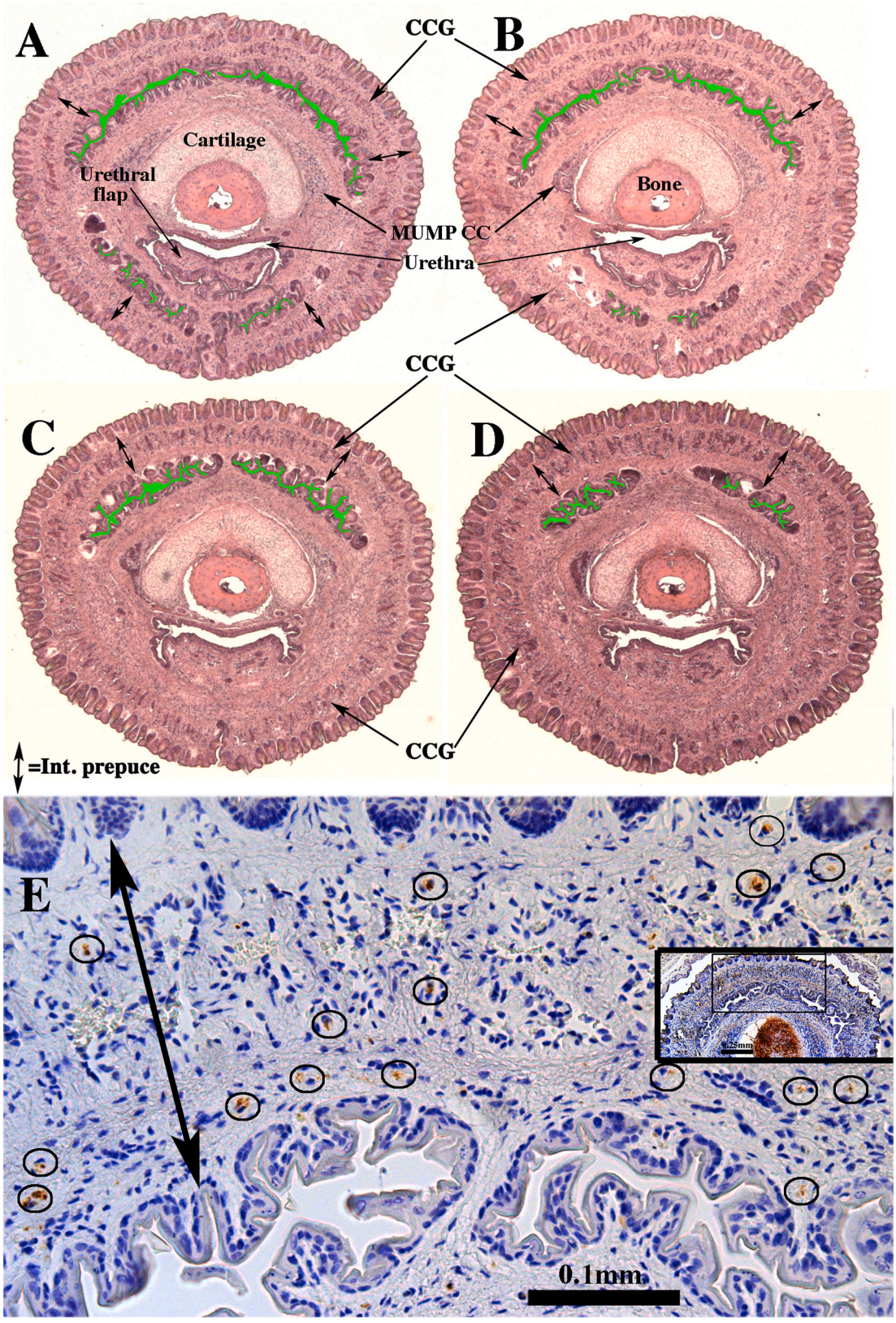

Fig. 8. Transverse sections of an adult mouse penis ordered from distal (A) to proximal (D). Given the position of the sections, the internal prepuce is nearing its proximal terminus and therefore is incomplete in C & D. The stromal wall of the internal prepuce is denoted by double-headed arrows, and the corpus cavernosum glandis is located in part within the stromal wall of the internal prepuce. The internal preputial space is denoted in green. (E) is a transverse section of an adult mouse penis through the internal prepuce containing the corpus cavernosum glandis immunostained with an antibody to S100 to reveal nerve fibers (outlined by circles) within the internal prepuce. Note low magnification inset. CCG = corpus cavernosum glandis, MUMP CC = MUMP corpora cavernosa. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

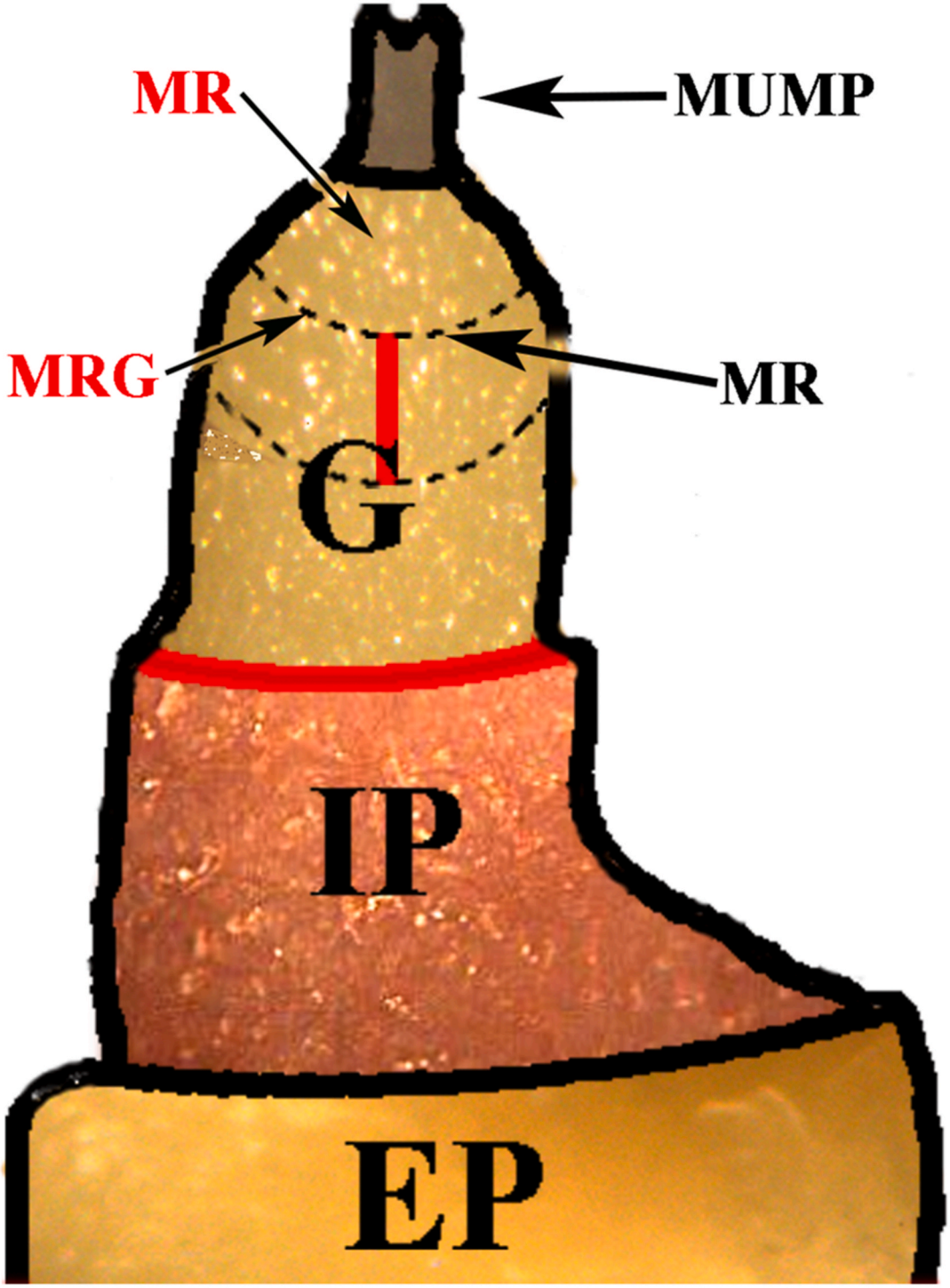

Fig. 9. An exact tracing of Fig. 1A of Phillips et al. (2015) artificially colored. The labels in black are from Phillips are: EP = the hair-bearing external prepuce, IP = internal prepuce; G = glans; MR = MUMP ridge, MUMP = male urogenital mating protuberance. Labels in red represent our interpretation. Note that the junction between the structures labelled G and IP is approximately at the midpoint between the distal edge of G and the EP. The dotted lines denote the position of the mouse internal prepuce is described by Blaschko et al. (2013), and the red bar represents its average maximal depth (616 mm, 117 SD, N = 7). We have added the red labels (MR and MRG [MUMP ridge groove] which conform to SEMs of the mouse penis as in Figs. 2 & 5A, this paper. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

becomes associated with the developing corpus cavernosum glandis (Fig. 6). Penile nerves are associated with the corpus cavernosum glandis within the stromal wall of the internal prepuce. The internal preputial lamina of the neonate is canalized in adulthood to define the internal preputial space. The depth of the internal preputial space varies circumferentially, being particularly shallow in the mid-dorsal position (Fig. 1), but with a maximal depth of ~600 μm. Accordingly, we consider it unlike that the internal prepuce protracts during adult penile erection. Thus, we provide considerable supporting anatomic detail for the internal prepuce integral to the mouse glans penis.

Because we have described in detail two prepuces in the mouse, external prepuce and internal prepuce, we are obliged to re-name the embryonic precursor of the preputial lamina as the external preputial lamina to distinguish it from the internal preputial lamina seen in the neonate (Fig. 6). The development of the preputial lamina (now designated as external preputial lamina) has been exhaustively described through fetal, neonatal and early pubertal periods by numerous investigators (see reviews by Liu et al., 2018 and Cunha et al., 2020). In the course of development the external preputial lamina canalizes in males in a distal to proximal direction (Cripps et al., 2019) to create a single continuous space (Mahawong et al., 2014b), which for decades has been called the preputial space, but is now appropriately re-named the external preputial space. The external preputial space begins distally at the meatus of the perineal appendage (external prepuce) and extends proximally to a blind cul-de-sac where the mucosa lining the external preputial space reflects onto the surface of the glans to become penile epithelium (Cunha et al., 2015; Mahawong et al., 2014b; Phillips et al., 2015; Rodriguez et al., 2011; Sinclair et al., 2016b). The epithelial lining of the external preputial space is (a) solely and exclusively derived from a single structure (the external preputial lamina), (b) begins distally within the external prepuce (the perineal appendage) and extends un-interrupted proximally to terminate by reflecting onto the surface of the glans. Accordingly, it is appropriate and justified to designate the continuous lining of the space housing the mouse penis the external prepuce and the space as the external preputial space. The descriptor, external, is appropriate to distinguish these terms (external prepuce and external preputial space) from an entirely different structure, the internal prepuce and internal preputial space integral to the mouse glans penis.

Mating (and we believe also urination) involves extrusion of the mouse penis through and beyond the meatus of the external prepuce (the hair-bearing perineal appendage). In the resting state the tip of the MUMP is located within the external preputial space (Hennefarth et al., 2020). During mating and thus penile erection, the glans extends distally beyond the meatus of the perineal appendage (external prepuce) to achieve intromission into the vagina. This is a complicated biomechanical process in mice (and also in rats) that involves both hemodynamic engorgement of erectile bodies as well as contraction of ischiocavernosus and bulbospongiososus muscles (Elmore and Sachs, 1988; Hart and Melese-D'Hospital, 1983; Hennefarth et al., 2020; Sachs, 1982). For successful mating it would appear that the urethral meatus of the glans penis must enter the vagina in order for semen to be deposited and coagulate into a vaginal plug. The penile urethral meatus is about 600–700 μm proximal to the distal tip of the MUMP. Thus. Successful mating may involve extrusion the glans penis beyond the perineal appendage (external prepuce) by ~2 mm, powered by the biomechanical forces generating erection (Hennefarth et al., 2020).

We speculate that urination in the mouse may also involve a similar biomechanical mechanism. The argument supporting this idea is a bit circular but merits consideration. Perinatal treatment with estrogens elicits ventral tethering of the glans penis of the mouse to the inner surface of the preputial lining (Blaschko et al., 2013; Mahawong et al., 2014a; Sinclair et al., 2016a, 2016b; Warner et al., 1979). This ventral tethering of the glans penis surely prevents distal extrusion of the penis. There are two urinary symptoms to this condition. Such male mice with penile tethering exhibit a perpetually wet and urine stained perineum, and preputial stones form within the preputial space (Mahawong et al., 2014a; Warner et al., 1979). The stones are composed of crystallized urine by chemical analysis (Warner et al., 1979). Our interpretation of these events is that during urination in normal mice, the penis is extruded beyond the preputial meatus so that urination can occur cleanly to the exterior. In mice with penile tethering, urine is expelled into the external preputial space and then subsequently dribbles out. Urine retained in the preputial space crystallizes to form stones. This speculation requires further examination.

In summary, the internal prepuce as described previously (Blaschko et al., 2013) and herein is integral to the mouse glans penis and comports with the definition of a prepuce (Cold and Taylor, 1999). It is innervated by penile nerves and contains an erectile body (corpus cavernosum glandis) known to play a critical role in erection (Hennefarth et al., 2020). The internal prepuce is associated with a space called the internal preputial space. The external prepuce of the mouse consists of the hair-bearing perineal appendage as well as its lining mucosa which extends continuously to its reflection onto the surface of the penis.

Acknowledgements

This study was supported in part by NIH grant K12DK083021.

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