

主論文

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1 **Dynamic Article**

2 Precise three-dimensional morphology of the male anterior anorectum reconstructed from large
3 serial histological sections: a cadaveric study

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5 **Running title:** 3D morphology of male anterior anorectum

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4 Study conception and design: Okada, Hasegawa, Shimokawa, Matsuda, Shinohara, and Sakai

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9 Final approval of the manuscript: All authors

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13

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15

1 **Abstract**

2 **Background:** Deep anatomical knowledge of the male anterior anorectum is important to
3 avoid urethral injury and rectal perforation in intersphincteric resection or abdominoperineal
4 resection for very low rectal cancer. However, its structure is difficult to understand because
5 the anorectum, muscles, and urogenital organs are complicatedly and three-dimensionally
6 arranged.

7 **Objective:** To revisit the anatomical information of the male anterior anorectum for
8 intersphincteric resection and abdominoperineal resection with a focus on the spatial muscular
9 morphology.

10 **Design:** Descriptive cadaveric study.

11 **Setting:** Ehime and Kyoto University.

12 **Patients:** Tissue specimens from nine male cadavers.

13 **Main Outcome Measures:** Specimens around the anterior anorectum were serially sectioned
14 in the horizontal, sagittal, or frontal plane; large semi-serial histological sections were created
15 at 250- μ m intervals. The series were stained with Elastica van Gieson, and some sections from
16 the series were studied by immunohistochemistry to detect smooth and striated muscles. Two
17 series were digitalized and reconstructed three-dimensionally.

Results: Two regions without a clear anatomical border were elucidated: (i) the anterior region of the external anal sphincter, where the external anal sphincter, bulbospongiosus muscle, and superficial transverse perineal muscle were intertwined; and (ii) rectourethralis muscle, where the smooth muscle of the longitudinal muscle continuously extended to the posteroinferior area of the urethra, which became closest to the anorectum at the prostatic apex level. Tight connection between the striated and smooth muscles was identified at the anterior part of the upper external anal sphincter and anterolateral part of the puborectalis muscle level.

Limitation: This study involved a small sample size of elderly cadavers.

Conclusion: This study clarified the precise spatial relationship between smooth and striated muscles. The detailed anatomical findings will contribute more accurate step-by-step anterior dissection in intersphincteric resection and abdominoperineal resection, especially with the transanal approach, which can magnify the muscle fiber direction and contraction of striated muscle by electrostimulation.

Introduction

Low anterior resection with intersphincteric resection or abdominoperineal resection is performed for very low rectal cancer. Negative circumferential resection margins and negative distal margins reduce the risk of local recurrence. However, abdominoperineal resection has a high rate of positive surgical margin and rectal perforation compared with sphincter-preserving surgery.^{1,2} One of the causes of this high rate is the difficulty of determining the dissection plane around the anterior anorectum, which contains the perineal body or the rectourethralis muscle (RUM).³ Recently, transanal total mesorectal excision has been gradually gaining popularity as a novel approach for low rectal cancer.⁴ Although magnified endoscopic visualization around the anorectum and the pelvic floor can be obtained, urethral or rectal injury in male patients has been recognized commonly by colorectal surgeons as one of the devastating complications.^{4,5} Thus, deep anatomical knowledge of the anterior anorectum, especially of men, is important to avoid urethral injury, rectal perforation, and positive surgical margins in abdominoperineal resection and intersphincteric resection.

The anorectum mainly consists of smooth and striated muscles; smooth muscles are the circular muscle layer (CM) and the longitudinal muscle layer (LM) of the rectal muscularis propria, and striated muscles are the external anal sphincter (EAS) and the puborectalis muscle (PRM),

which is the innermost part of the levator ani muscle.⁶⁻⁸ The detailed knowledge of the relationship between these striated and smooth muscles is important for abdominoperineal resection and intersphincteric resection,^{3,6} because these two-type muscles are distinguishable by their color, texture, and contraction by electrostimulation.⁹ However, at the anterior anorectum, in addition to the above smooth and striated muscles, perineal muscles, urogenital organs, and a fibromuscular mass and smooth muscle fibers, also known as the perineal body and RUM, respectively, are complicatedly and three-dimensionally arranged.^{10,11} Furthermore, the anatomical morphology or definitions of the perineal body and RUM are controversial, and these terms are often confused by anatomists, urologists, and colorectal surgeons.^{3,7,10-16} The confusion seems to be brought about by the differences in anatomical methodology, such as macroscopic observations during traditional anatomical dissection or microscopic observations by two-dimensional and focal histological examination alone.

Large serial histological sections containing the entire anterior anorectum and three-dimensional (3D) reconstruction would be required to correctly understand the spatial relationship between striated and smooth muscles of the male anterior anorectum. This study aimed to revisit the anatomical information of the male anterior anorectum for intersphincteric resection and abdominoperineal resection with a focus on the spatial muscular morphology.

Materials & Methods

Nine male cadavers (age range: 67-91 years) who had been donated to the Division of Anatomy and Embryology at Ehime University Graduate School of Medicine were used for this study.

This study was conducted following the Act on Body Donation for Medical & Dental Education law of Japan and the provisions of the Declaration of Helsinki. The protocol for this study was approved by the Ethical Committee of Ehime and Kyoto University.

Eight of nine cadavers were fixed by artery perfusion with 10% formalin, and the other cadaver was fixed using Thiel's embalming method.¹⁷ The pelvises were cut off at the level of the second sacral bone and the hip joint. These pelvises with the pelvic bones were placed into boxes filled with 15% gelatin and hardened at 4°C for 24-48 hours to preserve the positional relationship between the pelvic organs and pelvic bones.^{18,19} Then, the pelvises were frozen in a freezer at -20°C for 24 hours.

Nine pelvises embedded with gelatin were sectioned horizontally (3 pelvises), frontally (3), or sagittally (3) at approximately 5-mm intervals by a slicer (NF-385K or NFK-360, Nantsune, Osaka, Japan). The blade edge of this slicer was designed to have a sharp shape (not saw) to minimize tissue loss. After all sections were photographed for macroscopic examinations by a camera (EOS 7D MarkII, Canon, Tokyo, Japan), the specimens around the anterior anorectum

were dissected for histological examinations to fit Super Mega Slim Cassettes (CellPath, Powys, UK) that measured 75×50×5 mm.¹² The specimens were dehydrated in graded ethanol and xylene, and embedded in paraffin blocks.

The specimens were serially sectioned at 10 µm. Every 25th section was collected onto large glass slides measuring 76×52 mm and stained with Elastica van Gieson (EVG). Semi-serial histological sections with an interval of 250 µm were created. In addition, every 26th and 27th sections were collected onto glass slides. Some sections were stained using antibodies against smooth muscle actin (Actin, Smooth Muscle Ab-1, Clone 1A4, Thermo Fisher Scientific, Fremont, CA, USA) and skeletal muscle myosin (Myosin, Skeletal Muscle Ab-2, Clone MYSN02, Thermo Fisher Scientific) to detect the smooth muscle and skeletal muscle fibers, respectively.⁷ All slides were scanned as virtual slides using a slide scanner (BZX-700, Keyence, Osaka, Japan).

Two series of semi-serial histological sections stained with EVG were used for the 3D reconstruction. After manual registration of these series, smooth and striated muscles and urogenital organs were segmented while referring to the immunohistochemical sections using a 3D reconstruction software (Amira, Thermo Fisher Scientific).²⁰

Results

Histological observations of horizontal sections

Figure 1 shows the horizontal histological sections of the anterior anorectum from the anal side to the oral side. All slices of the semi-serial histological sections are shown in Supplemental Digital Content 1.

External anal sphincter (EAS) level

At the EAS level (Fig. 1A-F), the EAS covered two smooth muscle layers of the rectal muscularis propria: the circular muscle layer (CM) and the longitudinal muscle layer (LM). Some smooth muscle fibers of the LM were inserted into the EAS near the upper limit of the EAS (black arrowheads of Fig. 1E). The superficial transverse perineal muscle (STPM) was identified as the striated muscle bundle running from the posterior area of the ischiocavernosus muscle (ICM) toward the anterior part of the EAS. The thickness of this muscle had an individual morphological difference such as well or poorly developed types. Although the STPM fibers terminated to the rectourethralis muscle (RUM) above the EAS on the lateral side in only one of nine cadavers, the STPM fibers merged into the anterior part of the EAS in the other cases, and the border of both muscles was inseparable. Similarly, the bulbospongiosus muscle (BSM) was continuously attached to the EAS, and these striated muscles could not be

separated. Thus, the EAS, STPM, and BSM were intertwined with each other and formed the striated muscle complex (Fig. 1A-C). At the anterior part of the EAS, a fibromuscular mass often referred to as the perineal body, where these perineal muscles converged to, as described in anatomical atlases,^{21,22} was not identified.

Puborectalis muscle (PRM) level

At the PRM level (Fig. 1G-L), striated muscle fibers of the PRM sub-circumferentially covered the rectal muscularis propria from the anterolateral to the lateral and posterior aspect. At the anterior part, abundant smooth muscle fibers, which continued from the longitudinal muscle (LM), extended anteriorly. In this study, the successive smooth muscle containing elastic and collagen fibers was defined as the rectourethralis muscle (RUM). The RUM could not be separated clearly from the LM.

At the lower PRM level (Fig. 1G-I), the RUM communicated with the corpus spongiosum penis (CSP) and Cowper's gland (CG). Small striated muscle bundle extending laterally from the posterior part of the CG was identified (black arrows of Fig. 1I). We believe that this muscle corresponds to the deep transverse perineal muscle (DTPM) reported by previous studies.^{23,24} Although the thickness of the DTPM had an inter-individual morphological difference, it could

not be identified as a thick sheet-like striated muscle spreading laterally just anterior to the anorectum, as generally described in anatomical atlases.^{21,25} At the upper PRM level (Fig. 1J-L), the RUM extended to the posterior area of the urethral rhabdosphincter (RS), which was a striated muscle surrounding the urethra circularly.

At the anterolateral part, smooth muscle fibers from the LM were partially inserted into the PRM, and a tight connection between the smooth and striated muscles was formed (black arrowheads of Fig. 1H and 1K). This connection gradually became looser and changed into the pelvic fascia, which covered the surface of the PRM at the prostate level (white arrowheads of Fig. 1M and 1N).

Prostate level

At the prostatic apex level (Fig. 1M-O), the anterior extension of the rectourethralis muscle (RUM) disappeared and the anterior border of the longitudinal muscle layer (LM) became clear. Several nerve fibers, blood vessels, and adipose tissues were identified at the triangular area surrounded by the rectum, puborectalis muscle (PRM), and prostate. This triangular area corresponds to the neurovascular bundle (NVB) reported by Walsh.²⁶ However, the mesorectal fascia could not be clearly defined in adipose tissues at this level. Some nerves and blood

vessels penetrated the RUM and supplied the rectal muscularis propria, while others descended along the posterolateral and lateral sides of the urethral rhabdosphincter (RS) and supplied the RS and corpus spongiosum penis (CSP).

Histological observations of sagittal sections

Figure 2 shows the sagittal histological sections from the mid-sagittal to parasagittal sections. All slices of semi-serial histological sections are shown in Supplemental Digital Content 2. The urethra became closest to the rectal muscularis propria just inferior to the prostatic apex. At the mid-sagittal section, the rectourethralis muscle (RUM) occupied the posterior-inferior area of the urethra from the upper limit of the external anal sphincter (EAS) to the prostate apex (Fig. 2A-C). At the 3-mm parasagittal section, the RUM communicated with the urethral rhabdosphincter (RS) and Cowper's gland (CG) (Fig. 2D-F). At the 8-mm parasagittal section, the RUM fibers were partially inserted into the PRM (black arrowheads of Fig. 2G and 2H) and changed into the pelvic fascia of the PRM (white arrowheads of Fig. 2G and 2H).

3D reconstruction

Figures 3 and 4 show the left anterior oblique view and transanal view of the 3D reconstruction

images of the anterior anorectum, respectively. The multi-angle views of the segmented histological sections and 3D images are shown in Supplemental Digital Content 3. These 3D images can be used to visualize the spatial relationship between the smooth (blue) and striated (red) muscles, especially the three-dimensional extension of the rectourethralis muscle (RUM). The RUM, which was inseparable from the longitudinal muscle (LM), filled the space surrounded by the striated muscle complex, which consisted of the external anal sphincter (EAS), bulbospongiosus muscle (BSM), and superficial transverse perineal muscle (STPM), the urogenital organs of the prostate and urethra, and the bilateral slings of the puborectalis muscle (PRM) (Figs. 3B, 4C, and 4D). After division of the striated muscle complex in abdominoperineal resection or the separation at the intersphincteric plane between the EAS and LM in intersphincteric resection at the EAS level, both dissection planes always encounter the RUM at the PRM level. Since the urethra becomes close to the anorectum at the prostatic apex level as the 3D image shows (Figs. 3A, 3B, 4C, and 4D), surgeons have to be careful to avoid urethra injury during the division of the RUM.

Discussion

Large semi-serial histological sections and the 3D reconstruction images precisely clarified the spatial muscular morphology of the male anterior anorectum. The detailed descriptions of the relationship between striated and smooth muscles at each level will enable more accurate step-by-step anterior dissection in intersphincteric resection and abdominoperineal resection, especially with the transanal approach. Moreover, the 3D image can be used to visualize the 3D anatomical structure hidden behind the dissection plane by the multi-angle views and transparent organs, which is informative for surgeons so they can avoid unexpected complications.

Anatomical structure of the anterior anorectum at the external anal sphincter (EAS) level

The anterior structure of the male EAS has been generally described as striated muscle fibers of the EAS, bulbospongiosus muscle (BSM), and superficial transverse perineal muscle (STPM) that converge to the perineal body, which comprises the fibromuscular mass in anatomical atlases.^{21,22} However, such a fibromuscular mass could not be identified anterior to the EAS in this study. Instead, these striated muscle fibers were intertwined with each other and formed the striated muscle complex (Figs. 1A, 1C, and 4A). This finding is important

1 knowledge for the anterior dissection of abdominoperineal resection. Surgeons have to keep in
2 mind that there is no clear border between the STPM, BSM, and EAS. The STPM fibers, which
3 run transversally from the posterior ischiocavernosus muscle to the anterior EAS, should be
4 identified at both anterolateral parts of the EAS. Subsequently, the striated muscle complex is
5 divided while confirming the STPM fibers that have already been exposed at both anterolateral
6 parts.

7 At the anterior part of the upper EAS level, the smooth muscle fibers of the longitudinal muscle
8 (LM) were partially inserted into the EAS (arrowheads of Figs. 1E, 2E, and Fig. 4B) and
9 communicated cephalad with the rectourethralis muscle (RUM). This finding indicates that the
10 separation at the intersphincteric plane gradually becomes difficult as the upper EAS level is
11 approached. This connection should be sharply divided at the inside of the EAS, confirming
12 the contraction of the EAS by electrostimulation; subsequently, the RUM at the puborectalis
13 muscle (PRM) level will be encountered in the dissection plane.

15 ***Anatomical structure of the anterior anorectum at the puborectalis muscle (PRM) level***

16 In this study, we defined wide anterior extension of the smooth muscle, which continued from
17 the longitudinal muscle (LM), as the rectourethralis muscle (RUM) (Figs. 1H, 1K, 2B, 2E, 3B,

4C, and 4D). The RUM has been generally described as smooth muscles that interposed between the anorectum and urethra.^{7,14,27} The term RUM appears to be mostly established among urologists as a part where rectal wall injury tends to occur during perineal radical prostatectomy.^{13,14,16,27-29} Similarly, in intersphincteric resection or abdominoperineal resection, there are always risks of rectal or urethral injury during the division of the RUM due to the unclear border between the urethra and anorectum.

On the contrary, this part is also often called the perineal body, instead of the RUM.^{3,10-12}

Therefore, the definition of the perineal body is confusing and controversial, including the anterior part of the EAS as described above,^{13-16,27,28,30} the area between the urethra and anorectum,¹⁰ or the whole area below the prostate and perineal skin.^{3,11,12} In this study, the anterior anorectum consists of striated and smooth muscles, and a specific structure corresponding to the perineal body could not be distinguishable. Instead, Muro et al. described the perineal body as a region between the rectum, anal canal, and urogenital organs, which seemed to be reasonable to prevent terminological confusion.³¹

At the anterolateral part, the smooth muscle fibers from the LM were inserted into the puborectalis muscle (PRM) of the levator ani muscle (black arrowheads of Figs. 1H, 1K and 2H). The connection between the levator ani muscle and LM identified not only anterolaterally

1 but sub-circumferentially from the anterolateral part to the posterior part of the anorectum.^{6,32}

2 Additionally, especially at the posterior part, the thick smooth muscle bundle, the so-called

3 anococcygeal ligament or ileorectococcygeus muscle, extended from the LM to the superior

4 aspect of the coccyx.^{8,33,34} Shafik described these connections as the “hiatal ligament,” which

5 circumferentially filled the gap between the levator ani muscle and the anorectum or the

6 peripheral organs.³⁵ That is, the anorectum is sub-circumferentially fixed to the levator ani

7 muscle via the connection between the LM and the levator ani muscle, and it is connected to

8 urogenital organs via the RUM at the anterior part and to the coccyx via the anococcygeal

9 ligament or ileorectococcygeus muscle at the posterior part. These connections are the entrance

10 into the intersphincteric plane, and must be sharply divided when intersphincteric resection is

11 performed.

12 During the anterior dissection of abdominoperineal resection at the PRM level, at first, at the

13 anterolateral part, bilateral slings of the PRM are divided. There is no clear anatomical

14 landmark to divide the PRM. We believe that the division behind the STMP level would be

15 appropriate for standard abdominoperineal resection unless tumor involvement is suspected.

16 On the other hand, during intersphincteric resection, the tight connection between the LM and

17 PRM is sharply divided along the inside of the PRM, while confirming the contraction of the

PRM by electrostimulation. However, since this connection gradually changes into the pelvic fascia of the PRM (white arrowheads of Figs. 1M, 1N, 2G, and 2H), too wide separation along the RPM would lead to the wide exposure of the lateral aspect of the neurovascular bundle and the prostate. Contrarily, deviation of the dissection plane into the longitudinal muscle may lead to rectal perforation. In both abdominoperineal resection and intersphincteric resection, there is no anatomical border around the RUM at the anterior part, where the urethra becomes closest to the anorectum. Surgeons should keep in mind that division of the RUM always has the potential risk of urethral injury or rectal perforation. Palpation of a urinary catheter,³⁶ visualization of the urethra using a lighting stent,³⁷⁻³⁹ and observation of the dissection plane from the transabdominal and the transanal teams may be useful to avoid these complications.

This study had two limitations. First, most cadavers used in this study were elderly with degenerative changes caused by aging. Second, the study had a small sample size. Despite these limitations, we believe that large semi-serial histological sections are comparable to magnetic resonance imaging (MRI) obtained from patients with rectal cancer. In the future, we would like to create a precise 3D anatomical model of the pelvic floor that considers individual variations by using the patient's MRI for reconstruction, and to establish a safer surgical technique for intersphincteric resection and abdominoperineal resection by using this 3D model

1 for surgical planning and image-guided navigation.

Conclusions

Large semi-serial histological sections and 3D reconstruction enabled precise visualization of the spatial relationship between smooth and striated muscles of the male anterior anorectum. Two regions without a clear anatomical border were elucidated: (i) the anterior region of the external anal sphincter (EAS), where the striated fibers from the EAS, bulbospongiosus muscle (BSM), and superficial transverse perineal muscle (STPM) were intertwined, and (ii) the rectourethralis muscle (RUM), where the smooth muscle fibers from the longitudinal muscle continuously extended to the posteroinferior area of the urethra, which became closest to the anorectum at the prostatic apex level. During the division of the RUM at the prostatic apex level, surgeons have to be careful to avoid urethral or rectal injury. Furthermore, tight connection between the striated and smooth muscles was identified at two regions: (i) the anterior part of the upper EAS level, and (ii) anterolateral part of the puborectalis muscle level. We believe that these detailed anatomical findings will contribute to more appropriate anterior dissection during intersphincteric resection and abdominoperineal resection, especially with the transanal approach, which can magnify the muscle fiber direction and the contraction of striated muscle by electrostimulation.

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References

1. den Dulk M, Putter H, Collette L, et al. The abdominoperineal resection itself is associated with an adverse outcome: the European experience based on a pooled analysis of five European randomised clinical trials on rectal cancer. *Eur J Cancer*. 2009;45:1175-1183.
2. Fleshman J, Branda ME, Sargent DJ, et al. Disease-free survival and local recurrence for laparoscopic resection compared with open resection of stage II to III rectal cancer: follow-up results of the ACOSOG Z6051 randomized controlled trial. *Ann Surg*. 2019;269:589-595.
3. Stelzner S, Holm T, Moran BJ, et al. Deep pelvic anatomy revisited for a description of crucial steps in extralevator abdominoperineal excision for rectal cancer. *Dis Colon Rectum*. 2011;54:947-957.
4. Penna M, Hompes R, Arnold S, et al. Incidence and risk factors for anastomotic failure in 1594 patients treated by transanal total mesorectal excision: results from the international TaTME Registry. *Ann Surg*. 2019;269:700-711.
5. Rouanet P, Mourregot A, Azar CC, et al. Transanal endoscopic proctectomy: an innovative procedure for difficult resection of rectal tumors in men with narrow pelvis. *Dis Colon Rectum*. 2013;56:408-415.

6. Tsukada Y, Ito M, Watanabe K, et al. Topographic anatomy of the anal sphincter complex and levator ani muscle as it relates to intersphincteric resection for very low rectal disease. *Dis Colon Rectum*. 2016;59:426-433.
7. Nakajima Y, Muro S, Nasu H, Harada M, Yamaguchi K, Akita K. Morphology of the region anterior to the anal canal in males: visualization of the anterior bundle of the longitudinal muscle by transanal ultrasonography. *Surg Radiol Anat*. 2017;39:967-973.
8. Muro S, Yamaguchi K, Nakajima Y, et al. Dynamic intersection of the longitudinal muscle and external anal sphincter in the layered structure of the anal canal posterior wall. *Surg Radiol Anat*. 2014;36:551-559.
9. Batista-Lima FJ, Gadelha KKL, Oliveira DM, Vasconcelos TB, Brito TS, Magalhaes PJC. A simple laboratory exercise with rat isolated esophagus and stomach fundus to reveal functional differences between striated and smooth muscle cells. *Adv Physiol Educ*. 2017;41:291-297.
10. Oh C, Kark AE. Anatomy of the perineal body. *Dis Colon Rectum*. 1973;16:444-454.
11. Wu Y, Dabhoiwala NF, Hagoort J, et al. 3D topography of the young adult anal sphincter complex reconstructed from undeformed serial anatomical sections. *PLoS One*. 2015;10:e0132226.

12. Kralima AC, West NP, Treanor D, et al. The anatomy of the perineal body in relation to abdominoperineal excision for low rectal cancer. *Colorectal Dis.* 2016;18:688-695.
13. Zhai LD, Liu J, Li YS, Ma QT, Yin P. The male rectourethralis and deep transverse perineal muscles and their relationship to adjacent structures examined with successive slices of celloidin-embedded pelvic viscera. *Eur Urol.* 2011;59:415-421.
14. Porzionato A, Macchi V, Gardi M, Parenti A, Caro RD. Histotopographic study of the rectourethralis muscle. *Clin Anat.* 2005;18:510-517.
15. Uchimoto K, Murakami G, Kinugasa Y, Arakawa T, Matsubara A, Nakajima Y. Rectourethralis muscle and pitfalls of anterior perineal dissection in abdominoperineal resection and intersphincteric resection for rectal cancer. *Anat Sci Int.* 2007;82:8-15.
16. Brooks JD, Eggener SE, Chao WM. Anatomy of the rectourethralis muscle. *Eur Urol.* 2002;41:94-100.
17. Thiel W. The preservation of the whole corpse with natural color. *Ann Anat.* 1992;174:185-195.
18. Park JS, Chung MS, Hwang SB, Lee YS, Har DH, Park HS. Visible Korean human: improved serially sectioned images of the entire body. *IEEE Trans Med Imaging.* 2005;24:352-360.

- 1 19. Spitzer V, Ackerman MJ, Scherzinger AL, Whitlock D. The visible human male: a
2 technical report. *J Am Med Inform Assoc.* 1996;3:118-130.
- 3 20. Takashima Y, Terada M, Kawabata M, Suzuki A. Dynamic three-dimensional
4 morphogenesis of intrahepatic bile ducts in mouse liver development. *Hepatology.*
5 2015;61:1003-1011.
- 6 21. Netter FH. *Atlas of Human Anatomy.* 6th ed. Philadelphia: Saunders Elsevier; 2014.
- 7 22. Kopf-Maier P, ed. *Wolf-Heidegger's Atlas of Human Anatomy.* 6th ed. Basel: Karger; 2005.
- 8 23. Murakami G, Nakajima F, Sato TJ, Tsugane MH, Taguchi K, Tsukamoto T. Individual
9 variations in aging of the male urethral rhabdosphincter in Japanese. *Clin Anat.*
10 2002;15:241-252.
- 11 24. Nakajima F, Takenaka A, Uchiyama E, Hata F, Suzuki D, Murakami G. Macroscopic and
12 histotopographic study of the deep transverse perineal muscle (musculus transversus
13 perinei profundus) in elderly Japanese. *Ann Anat.* 2007;189:65-74.
- 14 25. Agur AMR, Dalley AF. *Grant's Atlas of Anatomy.* 12th ed. Baltimore: Williams &
15 Wilkins; 2009.
- 16 26. Walsh PC. Anatomical studies of the neurovascular bundle and cavernosal nerves. *J Urol.*
17 2005;174:566.

27. Soga H, Takenaka A, Murakami G, Fujisawa M. Topographical relationship between urethral rhabdosphincter and rectourethralis muscle: a better understanding of the apical dissection and the posterior stitches in radical prostatectomy. *Int J Urol*. 2008;15:729-732.
28. Myers RP, Cahill DR, Devine RM, King BF. Anatomy of radical prostatectomy as defined by magnetic resonance imaging. *J Urol*. 1998;159:2148-2158.
29. Matsubara A, Murakami G, Arakawa T, et al. Topographic anatomy of the male perineal structures with special reference to perineal approaches for radical prostatectomy. *Int J Urol*. 2003;10:141-148.
30. Aigner F, Zbar AP, Ludwikowski B, Kreczy A, Kovacs P, Fritsch H. The rectogenital septum: morphology, function, and clinical relevance. *Dis Colon Rectum*. 2004;47:131-140.
31. Muro S, Tsukada Y, Harada M, Ito M, Akita K. Spatial distribution of smooth muscle tissue in the male pelvic floor with special reference to the lateral extent of the rectourethralis muscle: Application to prostatectomy and proctectomy. *Clin Anat*. 2018;31:1167-1176.
32. Arakawa T, Murakami G, Nakajima F, et al. Morphologies of the interfaces between the levator ani muscle and pelvic viscera, with special reference to muscle insertion into the

anorectum in elderly Japanese. *Anat Sci Int.* 2004;79:72-81.

33. Courtney H. Anatomy of the pelvic diaphragm and anorectal musculature as related to sphincter preservation in anorectal surgery. *Am J Surg.* 1950;79:155-173.

34. Kinugasa Y, Arakawa T, Abe S, et al. Anatomical reevaluation of the anococcygeal ligament and its surgical relevance. *Dis Colon Rectum.* 2011;54:232-237.

35. Shafik A. Levator ani muscle: new physioanatomical aspects and role in the micturition mechanism. *World J Urol.* 1999;17:266-273.

36. Adamina M, Buchs NC, Penna M, Hompes R; St.Gallen Colorectal Consensus Expert Group. St.Gallen consensus on safe implementation of transanal total mesorectal excision. *Surg Endosc.* 2018;32:1091-1103.

37. Atallah S, Martin-Perez B, Drake J, Stotland P, Ashamalla S, Albert M. The use of a lighted stent as a method for identifying the urethra in male patients undergoing transanal total mesorectal excision: a video demonstration. *Tech Coloproctol.* 2015;19:375.

38. Okada T, Kawada K, Nakamura T, et al. A cadaveric demonstration of visualization of the urethra using a lighted stent during transanal intersphincteric resection. *International Cancer Conference Journal.* 2018;7:77-80.

39. Kawada K, Hida K, Yoshitomi M, Sakai Y. Use of a lighted stent to identify the urethra

1 during transanal abdominoperineal resection. *Tech Coloproctol.* 2018;22:467-468.

2

1 **Figure Legends**

2 **Figure 1. Horizontal histological sections**

3 Horizontal histological sections of a 73-year-old male cadaver showing Elastica van Gieson
4 (EVG), anti-smooth muscle, and anti-striated muscle staining from the anal side to oral side:
5 lower (A-C) and upper (D-F) EAS level, lower (G-I) and upper (J-L) PRM level, and
6 prostatic apex level (M-O). The striated muscle fibers (black dashed arrows of A) of the
7 BSM, STPM, and EAS are intertwined and form the striated muscle complex. The RUM,
8 which comprises smooth muscle fibers that continue from the LM, extends to the CG, CSP
9 (H), and RS (K) and contains the DTPM (black arrows of I) of the striated muscle. Smooth
10 muscle fibers are inserted into the striated muscle (black arrowheads of E, H and K), which
11 change into the pelvic fascia (white arrowheads of M and N).

12 BSM=bulbospongiosus muscle; CG=Cowper's gland; CM=circular muscle layer;
13 CSP=corpus spongiosum penis; DTPM=deep transverse perineal muscle; EAS=external anal
14 sphincter; ICM=ischiocavernosus muscle; LM=longitudinal muscle; NVB=neurovascular
15 bundle; Pr=prostate; PRM=puborectalis muscle; RS=rhabdosphincter; RUM=rectourethralis
16 muscle; STPM=superficial transverse perineal muscle; Ur=urethra

17

Figure 2. Sagittal histological sections

Sagittal histological sections of a 67-year-old male cadaver showing Elastica van Gieson (EVG), anti-smooth muscle, and anti-striated muscle staining: mid-sagittal sections (A-C), 3-mm (D-F) and 8-mm (G-I) parasagittal sections. The urethra becomes closest to the rectal muscularis propria just inferior to the prostatic apex (A-C). The RUM extends to the urethra and CSP (B), RS, and CG (E). Black arrows indicate the striated muscle of the DTPM (F). Smooth muscle fibers are inserted into the striated muscle (black arrowheads of D, E, G, and H), and change into the pelvic fascia (white arrowheads of G and H).

BSM=bulbospongiosus muscle; CG=Cowper's gland; CM=circular muscle layer; CSP=corpus spongiosum penis; DTPM=deep transverse perineal muscle; EAS=external anal sphincter; LM=longitudinal muscle layer; NVB=neurovascular bundle; Pr=prostate; PRM=puborectalis muscle; RS=rhabdosphincter; RUM=rectourethralis muscle; Ur=urethra

Figure 3. Left anterior oblique view of the 3D reconstruction image

The 3D image was reconstructed from horizontal semi-serial histological sections in Figure 1. (A) Peripheral 3D structure of the RUM. (B) Successive smooth muscle of the LM and RUM to A. (C) Striated muscle of the PRM and ICM to B.

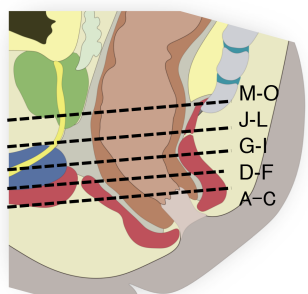
1 BSM=bulbospongiosus muscle; CG=Cowper's gland; CM=circular muscle; CSP=corpus
2 spongiosum penis; DTPM=deep transverse perineal muscle; EAS=external anal sphincter;
3 ICM=ischiocavernosus muscle; LM=longitudinal muscle layer; Pr=prostate;
4 PRM=puborectalis muscle; RS=rhabdosphincter; RUM=rectourethralis muscle; Ur=urethra

5

6 **Figure 4. Transanal view of the 3D reconstruction image of Figure 3**

7 Transanal view for the anterior dissection of intersphincteric resection or abdominoperineal
8 resection with the transanal approach. (A and B) Lower and upper EAS levels, respectively.
9 (C and D) Lower and upper PRM levels, respectively. (E) Prostatic apex level. The STPM
10 (black dashed arrows of A) merges into the anterior EAS. The smooth muscle fibers (RUM)
11 continue from the LM, extend widely to the anterior area, and are partially inserted into the
12 PRM at the anterolateral part (C and D).

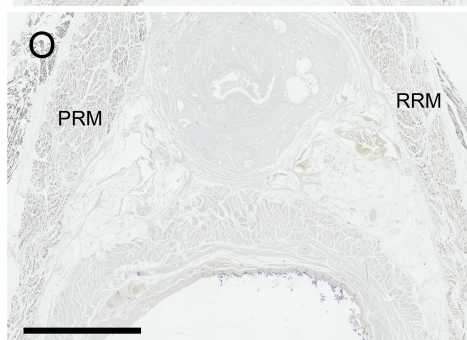
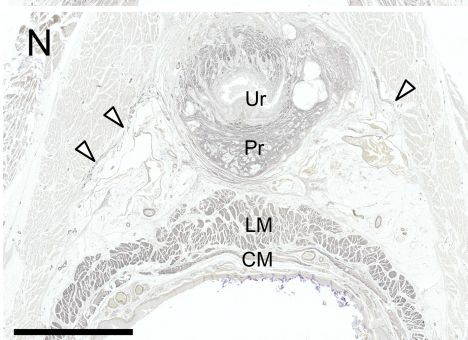
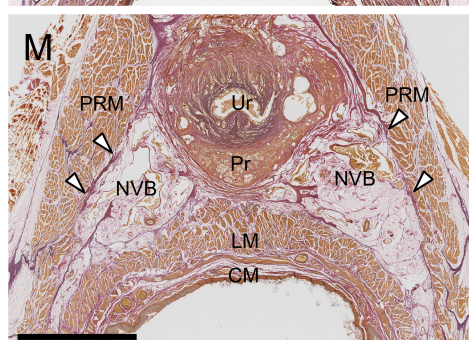
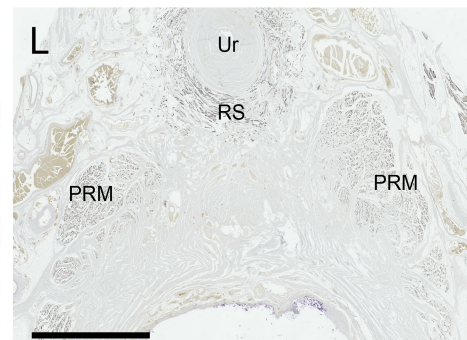
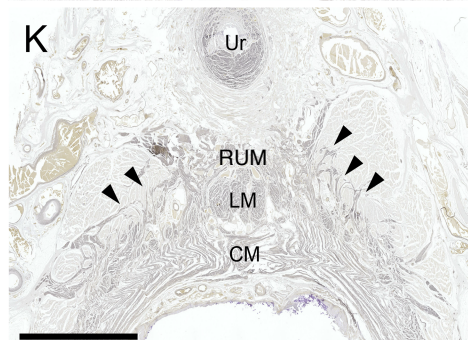
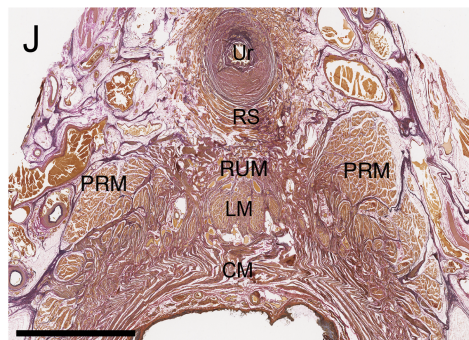
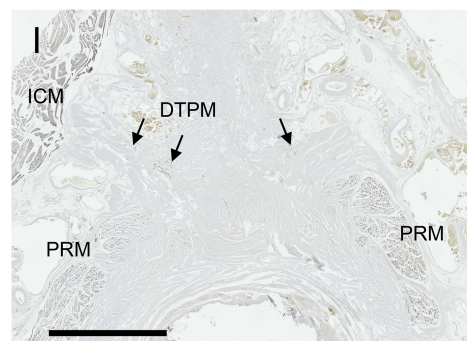
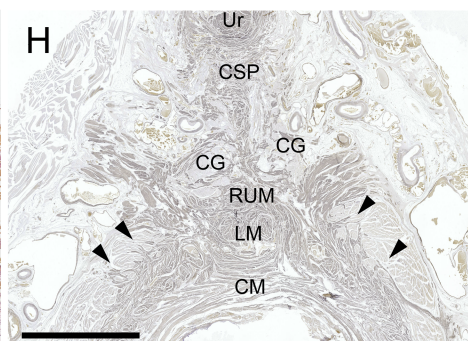
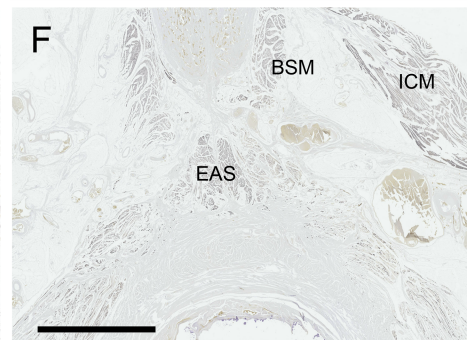
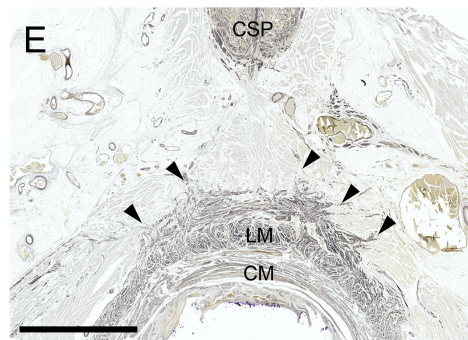
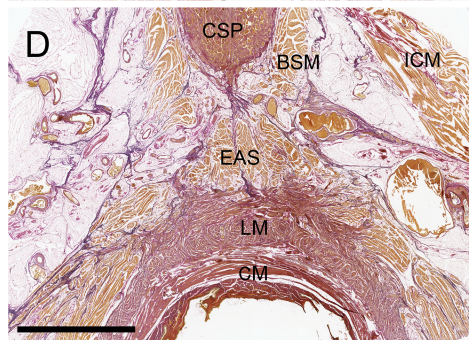
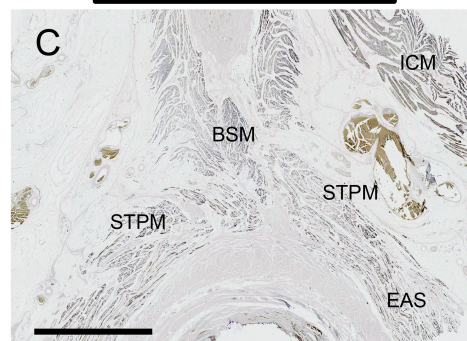
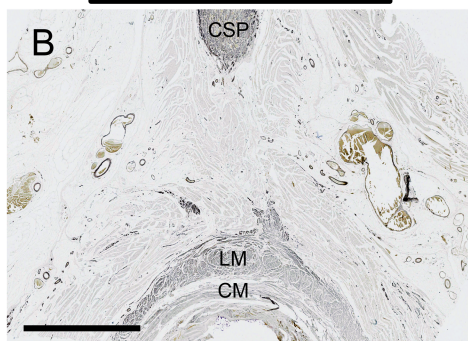
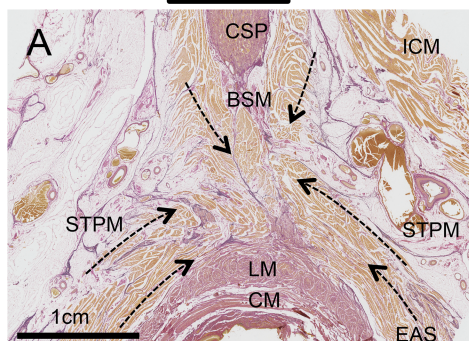
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14 spongiosum penis; DTPM=deep transverse perineal muscle; EAS=external anal sphincter;
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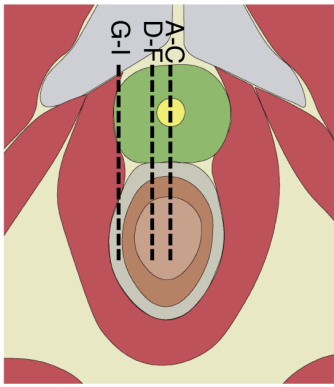


EVG

Smooth muscle

Striated muscle





EVG

Smooth muscle

Striated muscle

