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Morphological study of the arterial supply to the menisci in pigs with special reference to creating meniscus injury model

Yutaro Natsuyama et al., Nutrient artery around the stifle joint in pigs

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Abstract

Background: Some reports have used pigs to establish models of meniscus injury. However, accurate information on the origin, course, and access of the arteries supplying the menisci remains unclear. This information is important to avoid damaging vital arteries when creating the meniscus injury model.

Materials and methods: In this study, fetal and adult pigs were employed to investigate the arterial supply of the menisci in pigs using gross anatomical and histological methods.

Results: Macro-anatomically, the anterior horn, body, and posterior horn of the medial meniscus were found to be supplied by the medial superior genicular artery, medial inferior genicular artery, and posterior middle genicular artery. The anterior and posterior horns of the lateral meniscus were supplied by the cranial tibial recurrent artery and the middle genicular artery, respectively. Anastomosis was observed in some cases, but appeared to be infrequent and too thin to expect the anastomotic branches to provide adequate blood flow. The histological examination showed that the arteries entered the meniscus along the tie-fiber. The access process of the artery was the same irrespective of whether it was in fetal or mature pigs, the medial or lateral meniscus, or the anterior horn or body or posterior horn. The medial inferior genicular artery ran along the medial meniscus in the circumferential direction. Therefore, the clinical longitudinal incision should take into account the characteristics of the vessel course in order to protect the blood vessels from damage.

Conclusions: Based on the results of this study, the protocol for creating a pig meniscus injury model should be reconsidered.

Key words: arterial supply, stifle joint, medial meniscus, clinical anatomy, fetal and adult pigs

INTRODUCTION

Large animal models are important for the study of human etiology of acute injuries and chronic disease and for the development of new clinical applications for their treatment [6, 10, 26, 34]. Non-human primates are considered to more closely reflect human morphology than large animal models, but their usage is regulated due to ethical issues [15, 26]. In the field of musculoskeletal disorders, pigs are more commonly used as surrogates than other large animal models [7], and the pig meniscus has been used in meniscus injury models [11, 13, 18, 20]; however, the anatomical basis has been inadequate. The structure [3, 25, 33, 35], biomechanics [18, 27, 30, 32, 35], biochemistry [21, 29], and biology [5] have been studied, but the origin, course, and access process of the arteries supplying the menisci remain to be clarified.

Knowledge of the origin, course, and access of these supplying arteries could enable modeling that minimizes tissue invasion and better reflects the expected human outcomes. Although arterial supply is only part of the criteria used prior to selecting areas of invasion, the availability of such anatomical information will be useful to researchers in various scientific fields.

The purpose of this study was to structurally determine the origin, course, and access process of the pig meniscus nutrient arteries.

MATERIALS AND METHODS

Adult pigs and gross anatomy

Adult pigs (separated at hip height; average age: 200 days; n=12) were purchased from Nansyu Natural Pork (Kagoshima, Japan). Eight of the 12 sides were used for gross dissection, and 4 sides were used for histologic observation. For dissection, a 50-ml mixture of red ink (Xstamper; Shachihata Inc., Japan) and latex was injected into the femoral artery under pressure to clearly visualize the peripheral microarteries. The hind limbs were then immersion-fixed in a mixed fixative solution of 5% formalin and 50% alcohol. In addition to immersion fixation, a generous amount of the same fixative was injected into the deep tissues with a 20-ml syringe to ensure adequate fixation. At three weeks after immersion fixation, detailed dissection was performed around the stifle joint.

During dissection, the adipose tissue and superficial muscles were carefully removed to expose the arteries. The origin and course of the arterial supply in the menisci were then recorded.

Fetal pigs and dissection

Fetal pigs (n=20 sides; crown-rump length, approximately 30-35 cm) were purchased from Bio Corporation Company (Minnesota, USA) without any sex restriction. Fourteen of the 20 sides were used for gross dissection, and 6 were used for histologic observation. The supplier of the fetal pigs had already injected red latex into the arterial system and blue latex into the venous system, followed by preservation in 10% formalin solution [8]. The dissection procedure of the fetal pigs was similar to that of the adult pigs.

Preparation of tissue specimen

Tissue samples were collected from the medial and lateral stifle joints of the adult and fetal pigs. Medial tissue samples included the medial collateral ligament (MCL), joint capsule, and medial meniscus (MM). For the MCL, the femoral attachment was defined as the medial epicondyle of the femur and the tibial attachment was defined as the distal semimembranosus and proximal popliteus. Lateral tissue samples included the lateral collateral ligament, joint capsule, and lateral meniscus (LM). These samples were thoroughly washed under running tap water for four to five hours, then dehydrated and routinely embedded in paraffin using a tissue processor (Tissue-Tek® VIPTM5Jr; Sakura Finetek Japan Co., Ltd., Japan). Sections were cut and mounted on gelatin-coated glass slides. Four serial sections were prepared at 200-µm intervals. Slides were stained with hematoxylin-eosin (HE) or Masson's trichrome.

Hematoxylin-Eosin (HE) staining and Masson's trichrome staining

The HE staining procedure was performed as follows: (1) deparaffinization and rehydration; (2) hematoxylin staining for 10 min; and (3) eosin staining and dehydration for 5 min.

Masson's trichrome staining reagent (Muto Pure Chemicals Co., Ltd., Japan) was used to observe collagen fibers and blood vessels, respectively. The procedure was performed as described by Prabhath et al. [24], briefly: (1) deparaffinization and rehydration; (2) Weigert's Iron Hematoxylin Solution 1 and 2 for nuclear staining; (3) Acid Fuchsion Biebrich Scarlet Mixture for cytoplasmic staining; (4) phosphomolybdic-phosphotungstic acid for differentiating; and (5) Anilin blue for collagen fiber staining.

The sections were then imaged and photographed using an optical microscope. Images were merged using Adobe Photoshop Elements 9.0 (Adobe Inc., USA).

RESULTS

Origin and course of the MM nutrient arteries in fetal pigs

In all 14 sides, the major nutrient arteries of the MM typically originated from the medial superior genicular artery, medial inferior genicular artery, and middle genicular artery (Fig. 1).

The superficial femoral artery bifurcated at the middle third of the femur into the saphenous and popliteal arteries. In some cases (n=4/14), the saphenous artery branches, which supply most of the musculature, anastomosed around the anterior aspect of the popliteus muscle (Fig. 1A, B). After crossing the level of the stifle joint, the popliteal artery passed through the deep part of the popliteus muscle and branched off the cranial tibial arteries and caudal tibial arteries. The cranial tibial artery then branched off the cranial tibial recurrent artery, and the caudal tibial artery terminated on the posterior surface of the lower leg.

The medial superior genicular artery arose from the popliteal artery at a level above the femoral condyles. The medial superior genicular artery descended along the medial border of the vastus medialis muscle, passed deep anterior to the gracilis and semimembranosus muscles, proximal to the femoral attachment of the medial head of the gastrocnemius muscle, and supplied the femoral attachment of the MCL and the anterior horn of the MM (Fig. 1A, B). In some cases (n=6/14), the medial superior genicular artery was anastomosed to the medial inferior genicular artery (joint line branch) around the body of the MM (Fig. 1A, B).

The medial inferior genicular artery originated medial to the popliteal artery at the level proximal to the stifle joint (Fig. 1A, B). The medial inferior genicular artery passed deep into the medial head of the gastrocnemius and divided into the joint line branch and distal joint line branch. The joint line branch passed deep through the MCL to the MM, reaching proximal to the attachment of the semimembranosus and supplying the body of the MM (Fig. 1A, B). In some cases (n=7/14), the medial inferior genicular artery (distal joint line branch) was anastomosed to the medial inferior genicular artery (joint line branch) at the body of the MM (Fig. 1A, B).

A medial branch of the middle genicular artery arose as a short branch popliteal artery at the level of the stifle joint as a short branch and supplied the posterior horns of the MM (Fig. 1C, D).

Origin and course of the LM nutrient arteries in fetal pigs

In all 14 sides, the LM nutrient arteries typically originated from the cranial tibial recurrent artery (Fig. 2) and the middle genicular artery (Fig. 1C, D).

The cranial tibial recurrent artery, which arose from the cranial tibial artery, passed proximal to the common peroneal nerve and superficial to the fibula and then supplied the anterior horn of the LM (Fig. 2). In some cases (n=4/14), the lateral inferior genicular artery was anastomosed to the cranial tibial recurrent artery. Because a clear definition of the posterior tibial recurrent artery and the lateral inferior genicular artery could not be confirmed, in this study, all branches that passed through the deep level of the lateral head of the gastrocnemius muscle and that were more proximal than the stifle joint line were defined as the lateral inferior genicular artery.

A lateral branch of the middle genicular artery bifurcated from the popliteal artery at the level of the LM as a short branch and supplied the posterior horns of the LM (Fig. 1).

A part of the anterior horn and body of the LM did not receive direct access from the external arteries as it was blocked by the common tendon of the peroneus muscle and long digital extensor (Sisson, S. 1930), and popliteus muscles (Fig. 2C, D).

Origin and course of menisci nutrient arteries in adult pigs

In all eight sides, similar to the fetal pigs as mentioned above, the nutrient arteries of the MM typically arose from the medial superior genicular artery (anterior horn), medial inferior genicular artery (body), and middle genicular artery (posterior horn) (Fig. 3). The nutrient arteries of the LM typically originated from the cranial tibial recurrent artery (anterior horn) and middle genicular artery (posterior horn) (Fig. 4). The bifurcation was shifted rostrally in adult animals in comparison to fetal pigs. The following anastomoses were observed: saphenous artery - medial inferior genicular artery (distal joint line branch) (n=2/8), medial superior genicular artery - medial inferior genicular artery (joint line branch) (n=2/8), medial inferior genicular artery (distal joint line branch) - medial inferior genicular artery (joint line branch) (n=3/8), and lateral inferior genicular artery - cranial tibial recurrent artery (n=3/8).

Histologic findings of the menisci

The access process of the arteries at the posterior horn of the MM is shown in Figure 5. The middle genicular artery branched from the popliteal artery, entered the posterior horn of the MM, and then ran circumferentially (Fig. 5). This access process was the same for the MM, LM, and the anterior to posterior horn. In addition, the development of the menisci in fetal pigs was not fully completed and only a few circumferential bundles were found, while arteries along the circumferential bundles could be observed in adult pigs.

The access process of the arteries at the anterior horn of the LM is shown in Figure 6. The cranial tibial recurrent artery entered the meniscus through the tie fibers. The process of arterial entry into the meniscus was the same as in the MM, LM, and anterior to posterior horn. No external arterial access was observed in the posterolateral aspect of the lateral meniscus adjacent to the popliteus tendon.

DISCUSSION

In this study, we evaluated the origin, course, and access process of the nutrient arteries supplying the stifle meniscus in the pigs. This study provided a clearer perspective on the detailed arterial supply in pigs by performing gross anatomy and histologic studies.

Overall, the anterior horn, body and posterior horn of the MM were supplied by the medial superior genicular artery, medial inferior genicular artery and middle genicular artery, respectively. The anterior horn and posterior horn of the LM were supplied by the cranial tibial recurrent artery and middle genicular artery, respectively. In some cases, the lateral inferior genicular artery was anastomosed to the cranial tibial recurrent artery on the popliteus muscle. Although the lateral inferior genicular artery passed around the LM, it did not provide a direct branch to the LM because there was a popliteus muscle barrier between the two. Arterial anastomoses were observed around the stifle joint in pigs as well as in humans, but they were not considered to be thick enough or frequent enough to provide adequate blood flow in the event of main artery injury; thus, protection of the main artery is still considered important.

To our knowledge, no study has reported the origin and course of nutrient arteries in the pig meniscus. Macroscopically, Góes et al. [15] used computed tomography to investigate blood vessels of the entire body in pigs, but the hind limbs were only superficially described. On the other hand, microscopically, the presence of blood vessels in the meniscus has been confirmed by immunohistochemical staining [35]. The present study bridged the gap between the two studies and represents the first report showing the origin and course of the nutrient arteries to the pig menisci. Although previous studies have reported the creation of a pig meniscus injury model, the origin and course of the nutrient arteries of the pig meniscus remain unclear. Therefore, our study can be considered as an important reference for reconsidering the creation of the pig meniscus injury model and thus for human knee surgery. Certainly, the course of these arteries in the pig stifle was similar to that reported in the human knee. One important difference between the human knee artery and the pig stifle artery was the medial inferior genicular artery. The medial inferior genicular artery was divided into the joint line branch and the distal joint line branch. The joint line branch was the main artery of the body of the MM. The joint line branches of the medial inferior genicular artery were present in all pigs and it may be important for nutrition in the MM. Lazaro et al. [19] reported that the medial superior genicular artery also gave off joint line branches in 20 of 21 human cases, suggesting that there were also arteries at the joint line in the human MM. However, many of the reports did not describe the joint line branches in the MM [4, 11, 18, 22, 23]. The blood supply of the human meniscus has been described as originating from the medial inferior, lateral inferior, and middle geniculate arteries [14] or the medial, lateral, and middle geniculate arteries [2, 9, 28]. Thus, in the human knee joint, the meniscus is often described as being nourished by the rete articulare genus. Although anastomoses between arteries were also observed in this study, it should be emphasized that, as mentioned above, the major arteries were present in each region.

Differences in the height of arterial branches were observed between fetal and adult pigs; this may be due to the relative upward shift of branches as bone grows. Although there were differences in vascular course, the origin and access process of the major arteries were the same. Therefore, we believe that the findings of this study can be reflected not only in fetal and adult pigs, but also in piglets used as a model during the growth period.

When creating the meniscus injury model, it is important to note that the main artery coursed longitudinally in the anterior horn of the menisci, horizontally in the body, and with a short branch in the posterior horn. Meniscus injury models have often been created by injury to the anterior horn of the MM through a longitudinal incision in the long axis of the pig tibia [12, 13, 18, 20]. From the present study, it is therefore clear that that the likelihood of damage to the artery was very high.

Histological examinations revealed the access process of the nutrient arteries supplying the menisci. The access process was along the tie fibers, similar to that reported in the bovine meniscus [1]. Although the course of the arteries to the meniscus differed between the MM and LM, the access process to the menisci was the same in the MM and LM. The same was observed between fetal and adult pigs, the anterior horn, body, and the posterior horn, suggesting that the access process of the nutrient arteries along the tie fibers was common in the menisci.

CONCLUSIONS

In conclusion, through the anatomic and histologic evaluation of the pig stifle joint, we have determined the precise anatomic characteristics of the origin, course, and entry process of the meniscus arteries. The results of the present study suggest that the protocol for creating pig meniscus injury models should be re-evaluated.

Acknowledgements

This study was supported by an institutional fund from Tokyo Metropolitan University.

Conflict of interest: None declared

Abbreviations: CrTRA, Cranial tibial recurrent artery; CPN, common peroneal nerve; GLT, gastrocnemius lateral tendon; GMT, gastrocnemius medial tendon; LCL, lateral collateral ligament; LIGA, lateral inferior genicular artery; LM, lateral meniscus; LSGA, lateral superior genicular artery; MCL, medial collateral ligament; MGA, middle genicular artery; MIGA, medial inferior genicular artery; MM, medial meniscus; MSGA, medial superior genicular artery; PA, popliteal artery; PM, popliteus muscle;

PMT, popliteus muscle tendon; SA, saphenous artery; VL, vastus lateralis; VM, vastus medialis

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Figure 1. Photograph (**A**) and schematic representation (**B**) of the right stifle of a fetal pig seen from the inside. The MCL was removed to reveal the arteries passing through the MM. Photograph (**C**) and schematic representation (**D**) of the right stifle of a fetal pig viewed from the posterior coronal plane. Arrowheads point to the anastomotic branches of the saphenous and popliteal arteries. Scale bars: 3 mm in A and C.

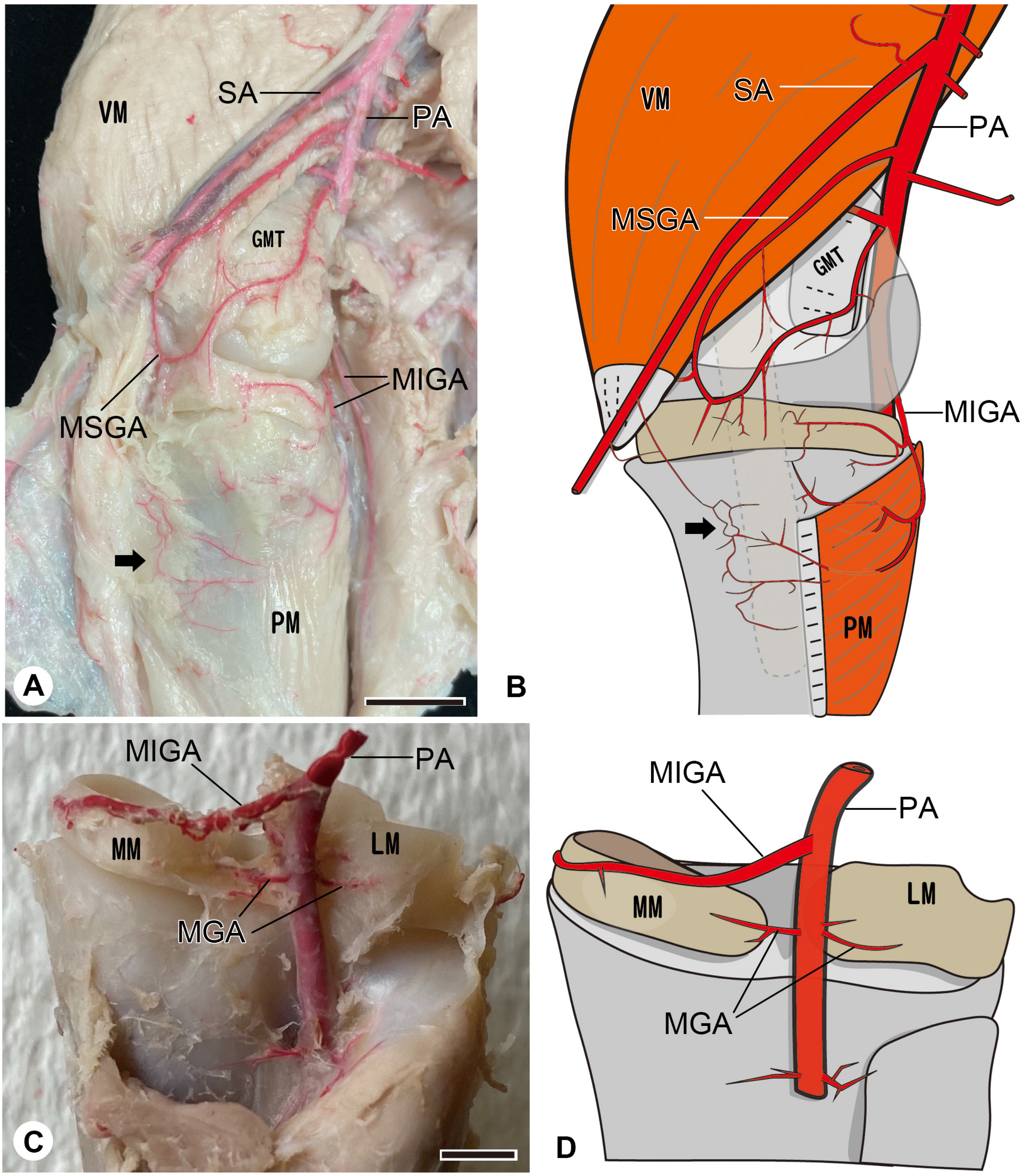
Figure 2. Photograph (**A**) and schematic representation (**B**) of the left stifle of a fetal pig seen from the outside. The lateral collateral ligament and the popliteus muscle tendon were removed to show the arteries of the LM (**C**). A schematic representation is shown in (**D**). The schematic representations of B and D show a slightly wider region to reveal the lateral superior genicular artery. *common tendon of the peroneus muscle and long digital extensor. Scale bars: 3 mm in A and C.

Figure 3. Photograph (**A**) and a schematic representation (**B**) of the left stifle of an adult pig seen from the inside. The MCL has been incised to show the arteries passing through the MM. The schematic representation in B shows a slightly wider region to reveal the artery origin. Scale bar: 10 mm.

Figure 4. Photograph (**A**) and schematic representation (**B**) of the left stifle of an adult pig seen from the outside. The schematic illustration in B shows a slightly wider area to reveal the lateral superior genicular artery. *common tendon of the peroneus muscle and long digital extensor. Scale bar: 10 mm.

Figure 5. (**A**): Histological image in a cross-section of the middle genicular artery and a horizontal section of the MM of a fetal pig with HE staining. (**B**): A shows an enlarged view of the area enclosed by the square “B” in A. (**C**): Extended 100 μm from B toward the tibia and located in the posterior horn of the MM. aMM — anterior medial meniscus; GM — gracilis muscle; GmM — medial head of the gastrocnemius muscle; pMM — posterior medial meniscus; PV — popliteal vein; T — tibia. Scale bars: 5 mm in A, 3 mm in B and C.

Figure 6. (**A**): Schematic representation of the right meniscus. The dotted line shows the lines of sections of B and C. (**B**): The anterior horn of the left LM of the adult pig was photographed in a cross-section, (**C**): histological imaging was performed with Masson's trichrome staining. ant — anterior; JC — joint capsule; pos, posterior; TF — tie fiber. Scale bars: 5 mm in B and C.



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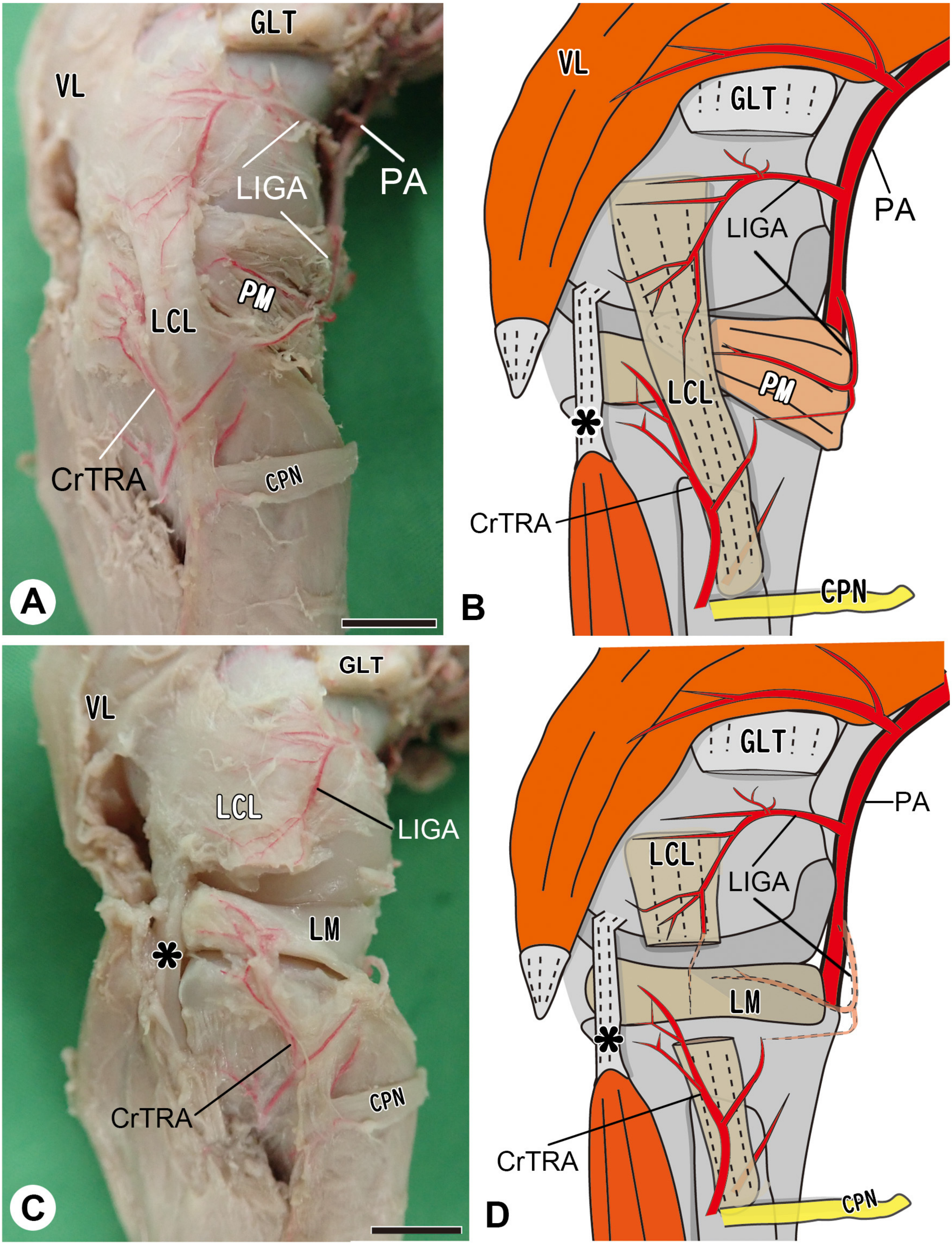
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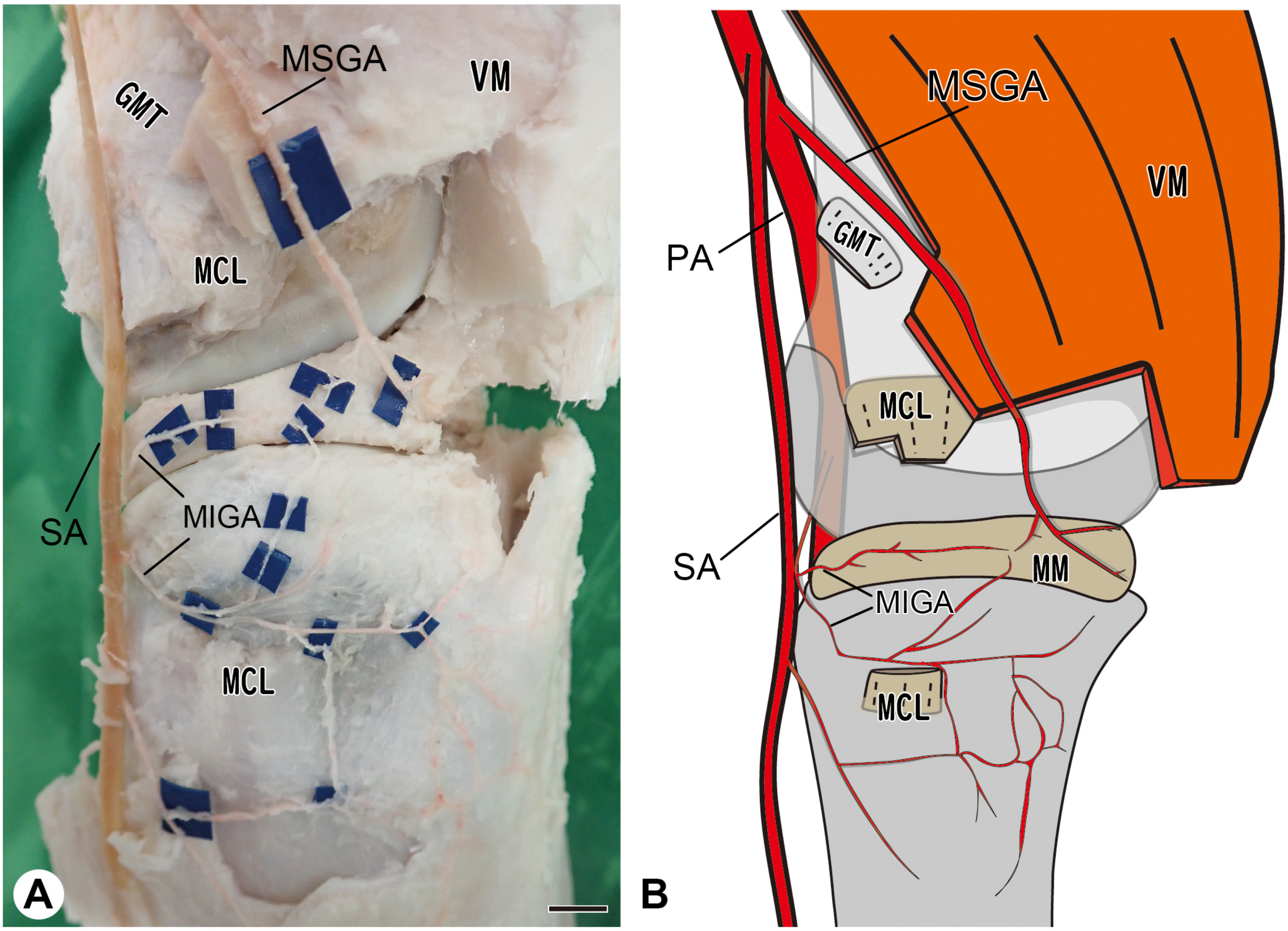

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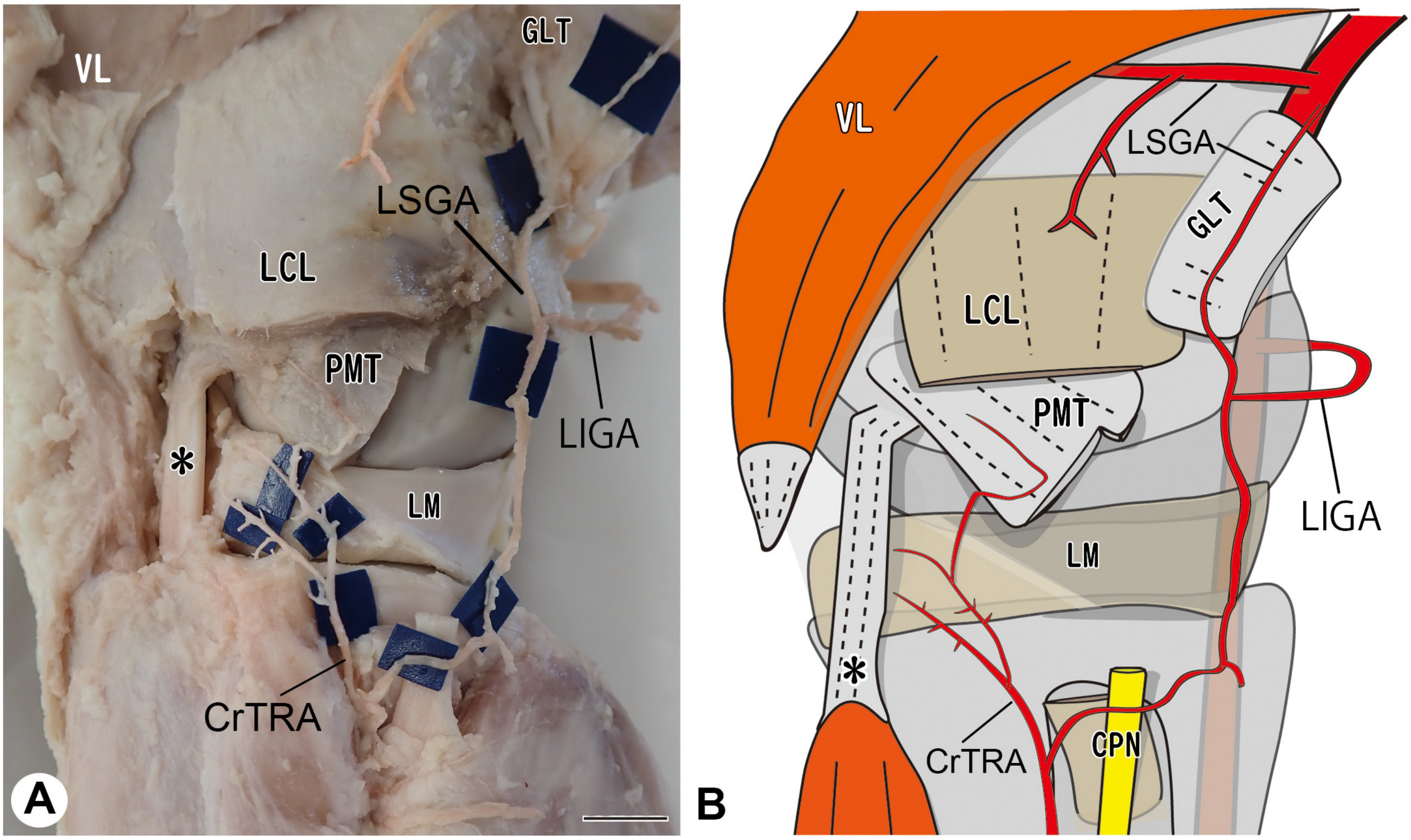
VL
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LSGA
LCL
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LM
CrTRA
A
B
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LM
*
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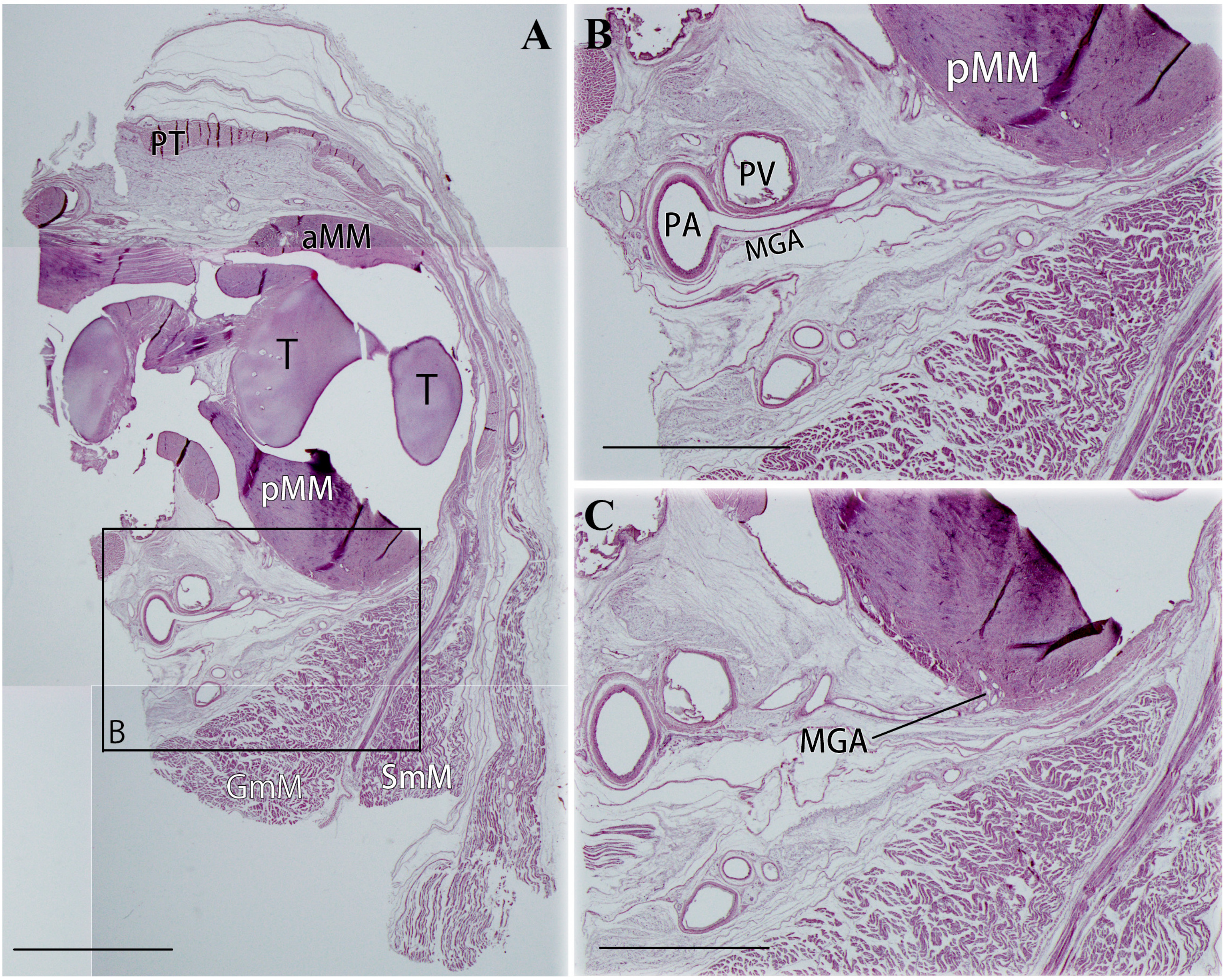
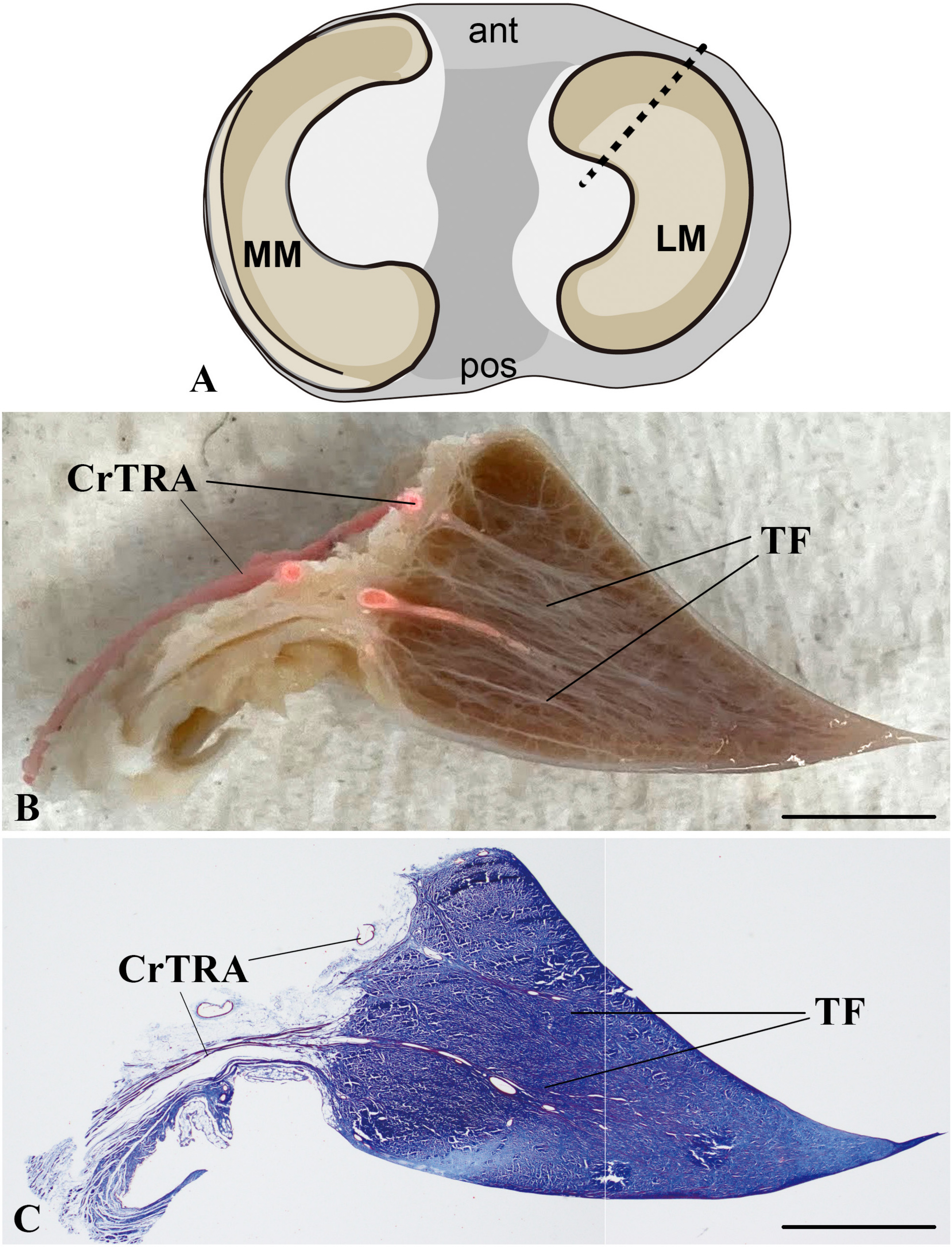
CPN



A
PT
aMM
T
T
pMM
B
GmM
SmM
B
pMM
PV
PA
MGA
C
MGA


ant
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LM
pos
A
CrTRA
TF
B
CrTRA
TF
C