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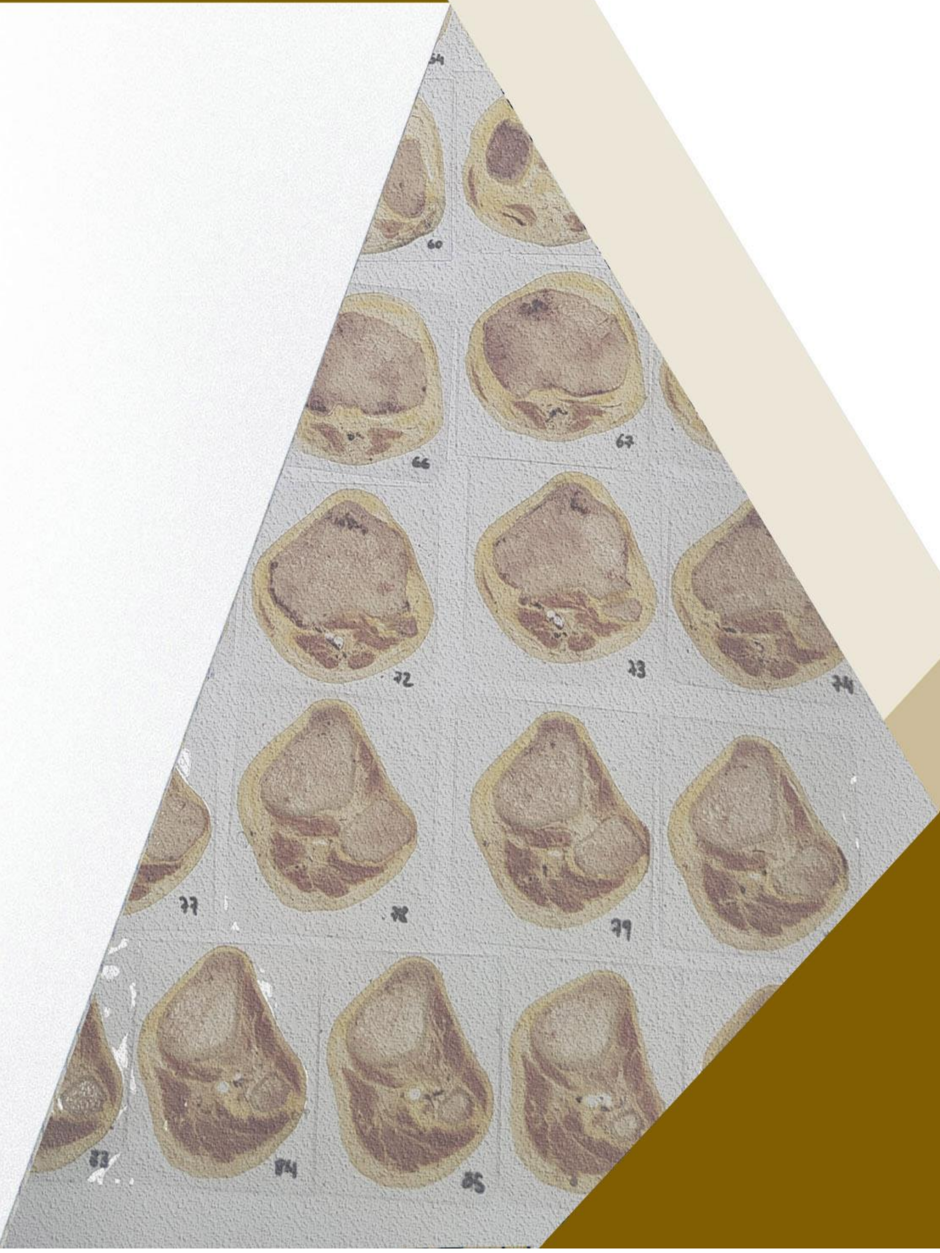
Anatomical description of the posterolateral corner of the knee

A descriptive study based on cadaveric dissection and E12 sheet plastination



A final undergraduate paper
by Marc Franco

Tutored
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Certifico que: el treball de final de Grau titulat "Anatomical description of the posterolateral corner of the knee. A descriptive study based on cadavèric dissection and E12 sheet plastination", presentat pel Sr. **Marc Franco Moral**, ha estat desenvolupat sota la meva direcció i compleix els requisits per a ser presentat i defensat.

I perquè així consti a tots els efectes oportuns, signo el present document a Girona a dia 6 de novembre de 2020.

Dr. Francisco Reina de la Torre

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Abbreviations

AC: articular cartilage

ACL: anterior cruciate ligament

BF: biceps femoris muscle

CH_2Cl_2; Me_2Cl_2; MeCl: dichloromethane

Cp: (joint) capsule

CPn: common peroneal nerve

Fe: femur

Fi: fibula

Gst: gastrocnemius muscle

ITT: iliotibial tract; iliotibial band

LCL: lateral collateral ligament; fibular collateral ligament

LM: lateral meniscus

mLCL: midthird lateral capsular ligament

PLC: posterolateral corner

PLm: peroneus longus muscle

PFL: popliteofibular ligament

PM: popliteus muscle

PT: popliteus tendon

Sm: soleus muscle

Ti: tibia

TP: tibial plateau

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Abstract

Background: major traumatisms affecting the knee usually associate posterolateral instability, which makes difficult the recovery of other lesions such as anterior cruciate ligament avulsion. The more anatomical the reconstruction of the posterolateral stabilisers the better biomechanical outcome is achieved. In order to provide an accurate diagnoses and treatment, basic knowledge must be pursued, being anatomy the first stage on a multi-specialist approach.

Objectives: to perform a precise description of the major stabilisers of the posterolateral corner of the knee, these being the lateral collateral ligament, the popliteus tendon and the popliteofibular ligament. A structure related to the capsule will be studied independently in order to determine its existence and components, defined by some as a midthird lateral capsular ligament or an anterolateral ligament.

Design: descriptive observational exploratory study, based on a series of cases.

Methods: macro and microdissection was performed on three fresh human knee specimens. E12 sheet plastination technique was applied to two more fresh human knees. Slices were then scanned with a 4K high definition scan for up-close study.

Results: all four structures were identified by dissection and also by sheet plastination. A description of each of them could be specified. Connexions between the popliteus tendon and the fibula have been demonstrated. Fibrous tissue compatible with the so-called midthird lateral capsular ligament was expressly reported.

Conclusions: combined use of macro-microdissection technique and E12 (Biodur®) sheet plastination have allowed us a better comprehension of the complex anatomy of the posterolateral compartment of the knee. Nevertheless, our sample value needs to be increased in order to corroborate our results and to evidence possible anatomical variations that may need to be taken into account.

Keywords: human anatomy, E12 sheet plastination, posterolateral knee compartment, lateral collateral ligament, popliteofibular ligament, midthird lateral capsular ligament.

Introduction / Justification

For at least a century and a half there has been a special interest in the study of the structures conforming the posterolateral aspect of the knee[1]. For the last years many authors have coincided on considering the posterolateral corner (PLC) of the knee as the "dark side"[2–6] of it, as knowledge about it is constantly evolving and authors do not seem to get to an agreement about the "gaps" its investigation leads to. PLC first recent description, being the one laid down in the *Nomina anatomica*[7], is non-exempt of polemic, as at least one of the structures that make up the PLC was omitted (as a contemporaneous author reported)[8] and lead to confusion for the next decades. This could explain the great amount of studies regarding the anatomy and biomechanics of the region that have been published recently on high impact anatomical, orthopaedic and radiological journals.

This area of study cannot be easily delimited, as its structures extend anteriorly up to Gerdy's tubercle of the tibia or beyond, and cover part of the posterior region with a deep relationship with the popliteus fossa. It would be a mistake to consider the anatomical region as the posterolateral quarter on a single axial vision, as the elements conforming it establish delicate and complex relationships with structures that cover the whole articulation (for instance the articular capsule or the patellar retinaculum)[9].

The specific interest in studying the PLC of the knee implicates both the functional importance and clinical impact of this area. Injuries that involve this complex result from traumatisms which associate high-enery[10], and so PLC's injuries are found on patients who also present other ligament tears. Injuries to the PLC which relate to chronic rotatory instability associate a tear in the anterior cruciate ligament and/or the posterior cruciate ligament on the 80% of the cases[11]. Anatomical description is becoming more relevant as a precise diagnoses of the lesions is gaining terrain in order to plan a specific surgical reconstruction. Both anatomical diagnoses and reconstruction are considered essential for a correct functional recovery and in the resolution of the posterolateral instability present in most subjects who have suffered a lesion in some of the structures of the posterolateral aspect of the knee[12]. Therapy starts with diagnosis, and precise diagnose is essential for a proper management of any lesion. Therefore, the more precise the anatomical description of the PLC the easier should be the orientation of treatment by an orthopaedic surgeon, radiologist or physiotherapist.

The PLC of the knee includes some anatomical structures that have been widely studied and others whose definition is controversial due to results published recently (**Table 1**).

	Primary stabilisers[6]	**Secondary stabilisers**[13]
Superficial layer		Iliotibial band Long head of the biceps femoris
Middle layer		Patellar retinaculum
Deep layer	Lateral (fibular) collateral ligament Popliteus tendon* Popliteofibular ligament* *the popliteus muscle, the popliteus tendon and the popliteofibular ligament have been described as a whole popliteus musculotendinous complex[6]	Lateral gastrocnemius tendon Mid-third lateral capsular ligament Coronary ligament Arcuate ligament Fabellofibular ligament Short head of the biceps femoris' tendon

Table 1 – Components of the posterolateral corner (PLC) of the knee classified according to layered anatomy proposed by Seebacher *et al*[14].

In a practical approach, the anatomical study of the PLC is useful and has modified the techniques of surgical reconstruction along time. The most popular reconstructive technique is the one described by LaPrade *et al*, which reconstructs individually each of the three main elements of the PLC, these being the lateral collateral ligament (LCL), the popliteus tendon (PT) and the popliteofibular ligament (PFL)[15]. When facing a lesion of the PLC, anatomic reconstruction is biomechanically superior to any non-anatomic method, although it does not exist (yet) any reconstruction technique capable to emulate the ligament forces and contact stresses under normal conditions[16]. It can be assumed that the more precise our knowledge on the anatomy of this area is, the better will be the outcomes achieved by any reconstructive technique.

The anatomy of the posterolateral corner (PLC) of the knee

Seebacher *et al*. proposed a characterisation of the posterolateral elements in three levels or layers, these being the superficial layer, the middle layer and the deep layer[14] (**Figure 1**). Description of the anatomical elements follows:

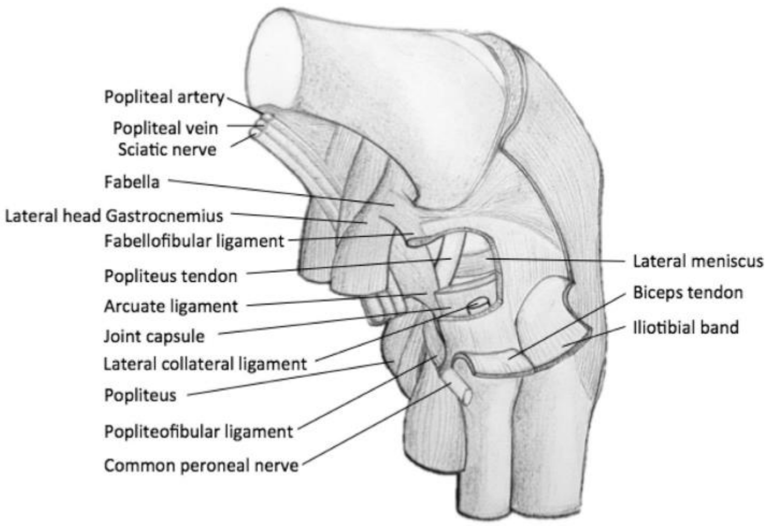


Figure 1 – Diagram presenting the main elements of the posterolateral corner and surrounding structures. Note how the midthird lateral capsular ligament is missing and the lateral collateral ligament has been sectioned for a better view of structures underneath. Reproduced from Maynard *et al*[17] according to conditions established by Copyright Clearance Center (RightsLink®), partner of Springer Nature Publications (See Addendum 2).

<u>Superficial layer: iliotibial tract and biceps femoris</u>

The superficial layer is found just below the skin and fat tissue. The iliotibial tract (or iliotibial band) is described in classical anatomic textbooks as a thickening of the fascia lata on the lateral side of the thigh[18] from which the lateral intermuscular septum origins (as it represents its insertion to the femur). It covers anteriorly the axis of flexion and is inserted laterally into the Gerdy's tubercle.

The biceps femoris is made up by two muscular bellies (short and long). Whereas the short head has little impact onto the PLC stability, the long head's tendon has a greater role both anatomically and biomechanically, partly due to its relationship with the distal end of the lateral collateral ligament[13]. It attaches to the apex of the fibular head. Whether it has its own insertion or merges with the LCL in a common attachment to the fibula is a matter of discussion.

<u>Middle layer: patellar retinaculum</u>

The middle layer consists solely of the patellar retinaculum. This structure is defined by vertical and oblique fibres that origin from the tendinous insertion of the vastus lateralis muscle and vastus medialis muscle. This fibrous structure attaches to the lateral and medial borders of the patella and covers part of the capsule. It has a minor role in PLC's stability. Classical descriptions of the joint capsule resemble the one we now relate to the patellar retinaculum, depicting different "reinforcement structures of the capsule" being the femoral aponeurosis, the quadricipital expansion and the patellae retinacula, both lateral and medial[19]. Nevertheless, these are distinct structures and must not be confused[9].

Deep layer: lateral collateral ligament, popliteus tendon, popliteofibular ligament and secondary stabilisers

The three major stabilisers of the posterolateral aspect of the knee are found under the patellar retinaculum, these being the lateral or fibular collateral ligament (LCL), the popliteus tendon (PT) and the popliteofibular ligament (PFL)[15].

The LCL is described as a fibrous cylindrical structure found attached to the lateral epicondyle of the femur and to the lateral aspect of the head of the fibula, on its distal third. It is considered as the primary varus-stabiliser of the knee[20]. As mentioned above, its relationship with the long head of the biceps femoris at its insertion is unclear. While some authors describe both structures conjoining in a common attachment[21], other publications defend they present different insertion sites and must be considered independent from one another[22].

The PT is considered independently from the popliteus muscle, although some authors describe the PT, the popliteus muscle and the PFL as a whole structure named popliteus musculotendinous complex[23,24]. The popliteus tendon goes intracapsular but stays extrasynovial[23]. It attaches on the lateral epicondyle, just under the attachments of the capsule itself, and its muscular body has its insertion on the popliteal fossa, on the posterior aspect of the knee. Classical descriptions of the popliteus muscle include a "meniscal origin" of the tendon, characterised into the posterior horn of the lateral meniscus[25]. Nevertheless, the exact interconnection between both structures is discussed, and a description in which the popliteus tendon does not relate to the meniscus has also been classically proposed[26].

The last main structure of the deep layer is a ligament that attaches the popliteus muscle (near to its musculotendinous junction) to the head of the fibula. Despite being described as a major stabilizer of the PLC[27], it has also been stated that this popliteofibular ligament would only play a major role in the posterolateral stability if there coexists a tear on the lateral collateral ligament[17]. Some of the descriptions of this structure coincide on defining the ligament as two-fasciculate, but even those disagree on their nature or disposition[17,29,30]. Its possible attachments to the capsule and even the lateral meniscus (through a lateral meniscotibial ligament)[31] are also a matter of discussion.

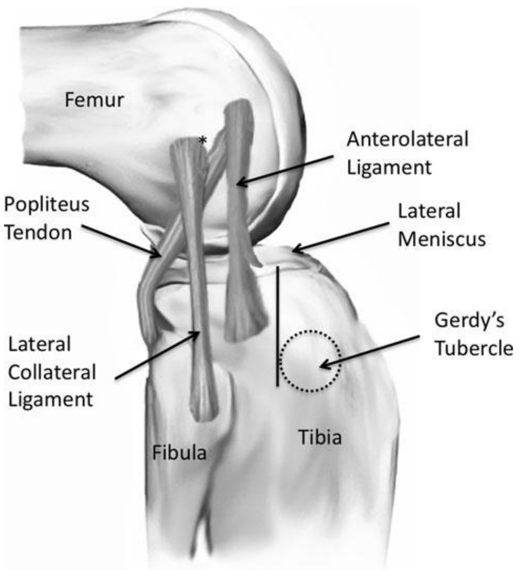


Figure 2 – this diagram presents the position of the hypothetical anterolateral ligament, understood as a thickening of the capsule. Reprinted by permission from Copyright Clearance Center RightsLink®: Springer Nature. *Knee surgery, sports traumatology, arthroscopy*. Vincent *et al*[28], 2012.

By its disposition, the secondary stabilisers can be classified as part of the deep layer. These structures include the lateral gastrocnemius tendon, the coronary ligament (which the posterior segment of the lateral meniscus to the tibia), the arcuate ligament, the fabellofibular ligament and the short head of the biceps femoris. Minor stabilizers of this region will not be addressed on this paper, because despite also rising a considerable amount of polemic, their clinical relevance has not been yet stated.

Nevertheless, a structure also described as a secondary stabiliser needs to be studied as its existence and characterisation are still in doubt, as it does not appear in classical anatomy books but has been recently described instead[21,32]. A thickening of the capsule can be found at the lateral aspect of the knee[33], and this finding has been described by different authors with different terms: anterolateral ligament[34] (**Figure 2**), meniscofibular ligament[35], lateral arcuate ligament[23], midthird lateral capsular ligament[36]… They all have been described as a thickening of or next to the lateral capsule. We decided to approach this lateral capsular thickening on the basis of this latter description of a midthird lateral capsular ligament, which attaches both to the femur and the tibia.

Consequently, classical descriptions of the elements present in the PLC are nowadays questioned by the scientific literature. Limits of the area, attachment sites and relationships between structures have been studied in an attempt to redefine the anatomy considering its possible relevance in knee's biomechanics. This study addresses the main differences signalled by modern literature and its intern disagreements.

Justification of the plastination technique

Systematic reviews of studies regarding the posterolateral corner of the knee focus on the clinical outcomes of knee injuries[37,38]. Methodological reviews for the anatomical study of the region are not available, and so it is not easy to determine which approaches have been taken into the description of the anatomical region. Although it cannot be precisely concluded, the natural evolution of the anatomical study regarding this location seems to start on anatomical dissection, followed radiological imaging (including MRI and CT) and recently with the addition of histological studies of specific elements of the PLC, or other techniques such as direct arthroscopic observation. Taking into account that the structures studied are extremely closely related, and can be confused or mixed up at plain sight, anatomical dissection could not be the best approach for this region. The extension of the region makes it difficult to use conventional histologic techniques in its study. Nevertheless, this approach has been proposed in some studies with foetal population, although their results are not precise[5].

The present study introduces a technique that although used in many publications regarding the musculoskeletal tissue has never been applied to the study of the PLC of the human knee, to the best of our knowledge. In addition to a microdissectional study with magnifying glass of three lower limbs, two knees have been processed with the E12 (Biodur®) sheet plastination. This technique has been previously used in the study of the neurovascular structures of the medial aspect of the knee[39], and proved to be useful in the anatomical study of the joint.

E12 sheet plastination can be considered a midpoint between the gross dissection and the histological preparation, as it offers the researcher vast accuracy of studied elements, especially connective tissue, but maintains the original anatomical relationships, as the shrinking is way minor than the one produced by histological means[40]. It consists in the plastination (term used to identify the technique which permits the preservation of tissues by replacing its water content by a polymerasable resin[41]) of sequential slices of an anatomical region, following a desired axis (coronal, sagittal, transverse or oblique) or a structure of interest. This permits the study of every element independently but also considering and preserving the structures surrounding it.

The role of laminar or cross-section anatomy has been widely used to better understand topographical anatomy when classical descriptions were not conclusive[42]. Semithin sheets/slices have been used in the interpretation of images obtained via CT or MRI [43–45], and students and residents trained with this anatomical approach are proven to perform better when confronting a diagnostic image analysis[46,47].

More recently, use of sheet plastination technique has been applied to three-dimensional reconstruction technology[48] by superposition of the slices. 3D reconstruction could help understanding areas of study for residents or guide surgeons during their operations[49,50], and may even modify existing surgical techniques[51]. The objectives of this study do not include three-dimensional reconstruction, as it could imply performing other investigations based on computational reconstruction. Nevertheless, a quite simple reconstruction will be added as a sample in the "Limitations of the study and future directions" section, presenting the wide possibilities of this approach.

Hypothesis and objectives

Hypothesis: *"combined use of both microdissection and E12 (Biodur®) sheet plastination techniques in the study of the posterolateral corner of the knee provides a new approach to the present anatomical description of the region"*.

Therefore and previously justified, the main objective of this study is resumed as:

- **To precise the description** of the posterolateral corner of the knee anatomy focusing on the *knowledge gaps* or incongruences described in the literature.

From this objective, four independent sub-objectives are presented, derived from the mentioned focused subject:

- **To precise the description** of the anatomical relationship between the biceps femoris muscle's tendon and the distal attachment of the lateral collateral ligament.
- **To precise the description** of the anatomical relationship between the popliteus tendon and its surrounding structures, with specific interest in the lateral meniscus.
- **To precise the description** of the components of the popliteo-fibular ligamentous fibres.
- **To determine the existence** of a lateral structure related or not to the capsule compatible with the *lateral midthird capsular ligament*.

Materials and methods

This project has been performed entirely in the Laboratory for Human Anatomy of the Clinical Anatomy, Embryology, Neuroscience Research Group (NEOMA) from the Medical Sciences Department of the University of Girona.

Cadaveric materials

For the development of the present work a total of five knees have been used. The anatomical samples were obtained from three donated cadavers belonging to the Body Donation Program of the University of Girona, managed by the Human Anatomy Area of the Faculty of Medicine. Ages of the specimens were comprised between sixty-two and eighty-four years old (**Table 2**). None of the subjects presented surgery antecedents on the knee area and any of the knees had been used in previous studies.

Ethics

This work contemplates the provisions of the Declaration of Helsinki in 1995 (revised in Brazil in 2013). All the specimens came from the voluntary Body Donation Program of the University of Girona. This program complies with the legal and ethical framework governing body donation procedures in our country. Furthermore, it is in accordance both with the International Federation Association of Anatomists (IFAA) guidelines[52] and with the Spanish Anatomical Society recommendations[53]. Thus, cadaveric materials for this study were obtained from individuals who donated in life their bodies to the benefit of research and university teaching (Annex /Addendum 1). Conditions, legal issues and ethics can be consulted on the University's website[54].

Technical details

Samples used in this project had been preserved differently according to the procedure they were set aside for. To perform the microdissectional study, three specimens were preserved by freezing at -25ºC (248K) after intravascular injection of coloured natural latex for a better identification of the vascular tree. Specimens destined to the sheet plastination study were preserved at -80ºC (193K) and no latex injection was performed.

Specimen	Left/Right	Sex	Age	Injection	Preserving technique	Use
Leg I	Left	Male	62 yo	Yes	Frosting at -25ºC (248K)	Macro and micro dissection
Leg II	Right	Male	62 yo	Yes	Frosting at -25ºC (248K	Macro and micro dissection
Leg III	Right	Female	84 yo	Yes	Frosting at -25ºC (248K	Macro and micro dissection
Leg IV	Right	Male	75 yo	No	Frosting at -80ºC (193K)	E12 (Biodur®) sheet plastination
Leg V	Left	Male	75 yo	No	Frosting at -80ºC (193K)	E12 (Biodur®) sheet plastination

Table 2 – technical details of specimens used in this project.

Anatomical study by macro and microdissection

Cadaveric dissection and microdissection of the posterolateral compartment was performed through a surgical microscope (KARL KAPS ® SOM 62 G-Nr 18406) on fresh (not fixed) legs, from which knees I, II and III were obtained.

Before microdissection, the vascular tree of specimens was injected with coloured natural latex. The technique is performed by the injection of a mixture of liquid latex (black coloured) through a catheter secured proximally in the femoral artery. The artery will be exposed by meticulous dissection, and the catheter should be attached with surgical thread. The injection must be pulsating, using a 10cc syringe. To prevent small vessels from breaking and ensuring their dye, injection must keep a constant and low pressure[55]. Injection is complete when extravasation of the liquid is observed on the toes capillaries, where previously an incision has been performed with a scalpel. In case the extravasation falls through, injection must be stopped when resistance to the pulse increases considerably. The injection process must continue even if there is an extravasation through the femoral vein, at a proximal level. This signals free passage and dye of the major vessels, but does not ensure that the treatment is reaching smaller vessels or capillaries. Experience shows to be recommended to put a clamp on the vein and to keep on with the artery injection.

Once injection is complete, the limb will be frozen for at least 48 hours for the dye to set.[55] Previous to the dissection the preparation must be unfrozen. Room-temperature defrosting is recommended, although in order to better preserve the tissues the preferred thawing temperature would be 2ºC (275K)[56], what we consider a refrigeration temperature.

High definition photography is used to document dissection findings in all of its stages. The camera used is a NIKON D5100, with Nikkor 18-200mm and Nikkor 69mm lenses. Images have been corrected computationally (program used was Adobe® Photoshop CC 2019[57]).

<u>Dissection procedure of the posterolateral corner</u>

<u>*Starting the dissection: skin and subcutaneous tissue*</u>

An operation window was opened on the lateral aspect of the knee, fifteen centimetres proximal and ten centimetres distal to the head of the fibula (which can be identified by palpation).

<u>*Identifying the structures below the crural fascia*</u>

Once the fat tissue is moved away, the crural fascia must be cut and retracted, presenting deep structures. At this point, the superficial stabilisers of the posterolateral corner can be identified (long head of biceps femoris and iliotibial tract). The lateral collateral ligament can be perceived under the tissue between these muscles, but cannot be seen.

Figure 3 – general view of the posterolateral aspect of a left knee.

Muscles and neurovascular structures located near the posterolateral corner are identified: peroneus longus muscle, extensor digitorum longus muscle, plantaris muscle and lateral head of the gastrocnemius muscle, tibial nerve and common peroneal nerve, lateral sural cutaneous nerve and saphenous vein (**Figure 3**).

Hoisting of the biceps femoris and retraction of the iliotibial tract

Figure 4 – the long head of the biceps femoris is sectioned and hoisted to present its relationship with the lateral collateral ligament. Left knee.

Next step includes sectioning of the biceps femoris muscle proximal to the lateral epicondyle of the femur. It will be retracted distally to unveil its intern face and its tendinous junction to the fibular head. At this point we can observe the lateral collateral ligament uncovered. The popliteus tendon is also visible, partly covered by the joint capsule and the gastrocnemius muscle (**Figure 4**).

The iliotibial tract is then retracted medially, in order to uncover the joint capsule. Vastus lateralis muscle is sectioned up to the window's proximal limit.

Other structures are also visible: popliteus vein, popliteus artery (from which sprouts the lateral superior and inferior genicular arteries) and the capsule itself.

Dissection of the superficial layer of the biceps femoris' muscle

The tendon of the long head of the biceps femoris is identified and differentiated from the lateral collateral ligament. They are separated to make visible the unaltered path of the lateral collateral ligament and its insertion **(Figure 5**).

Figure 5 – the tendon of the long head of the biceps femoris is differentiated from the lateral collateral ligament. Left knee.

Deep dissection: popliteus relations and attachments

Next step includes disinsertion of the popliteus muscle from the tibia. It will then be stretched and everted proximally in order to display connective tissue between the tendon and the fibula's head (**Figure 6**).

Figure 6 – the popliteus muscle is disinserted from the tibia and is retracted proximally to show its connections with surrounding structures. Left knee.

Inside-out dissection

To identify the capsular connections with the popliteus tendon and the lateral meniscus, an inside-out approach is proposed. A sagittal cut will be performed along the midsagittal plane of the whole specimen (**Figure 7**).

Pictures will be taken from the medial face of the lateral portion of the capsule. To prevent it from covering the posterior attachments and structures from an antero-medial vision, the lateral condyle of the femur is sawed off.

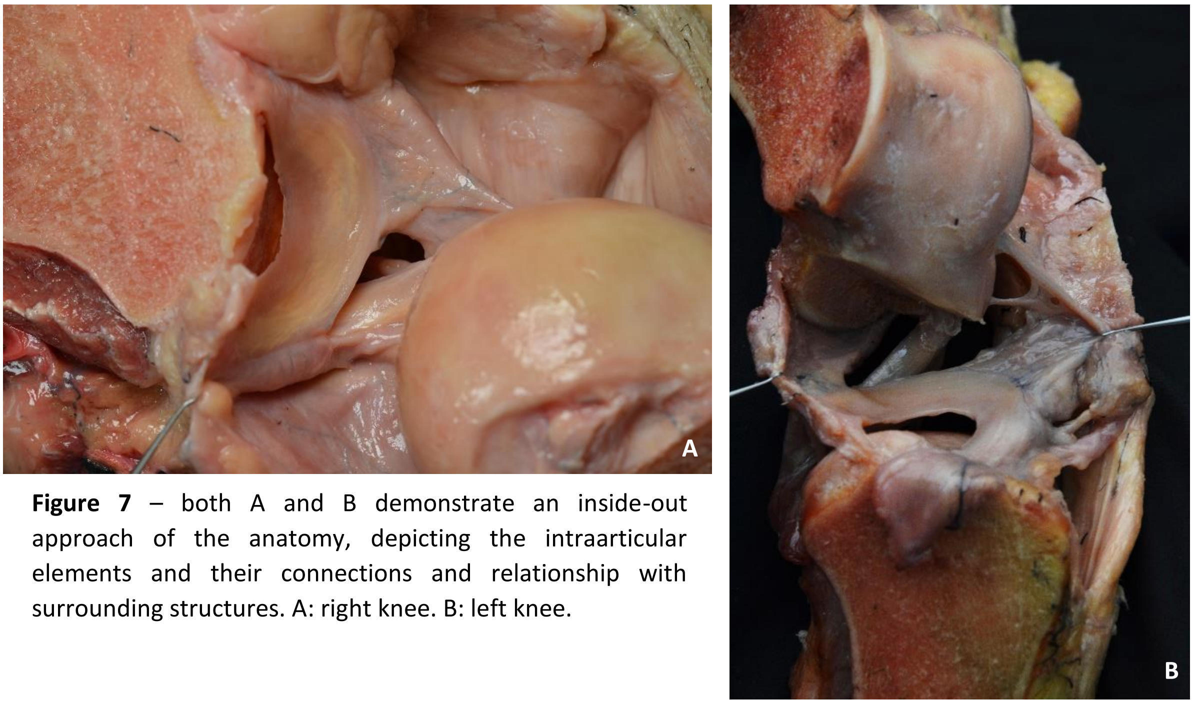


Figure 7 – both A and B demonstrate an inside-out approach of the anatomy, depicting the intraarticular elements and their connections and relationship with surrounding structures. A: right knee. B: left knee.

Anatomical study of the PLC by sequential slicing with E12 (Biodur®) sheet-plastination technique

Knees IV and V were processed using the E12 (Biodur®) sheet-plastination technique. E12 is a polymerisable epoxy resin, registered and commercialised by "BIODUR® GmbH Products & Services, Polymers, Equipment & Auxiliaries for Plastination" (Heildelberg, Germany). It is used for preservation of semithin anatomical sections between 1-3 millimetres thick.

Sheet-plastination technique consists of five stages or steps: specimen preparation, dehydration, degreasing or defatting, forced impregnation and curing[58–61]. The whole process will take at least 17 days (**Table 3**). Due to the volume of slices the process had to be doubled but could be partially overlapped. It took 33 days from start to finish obtaining, process and scanning all the slices. We obtained a total amount of 110 semithin anatomical slices, from which five had to be discarded as they presented irreparable structural damage that made them useless for our study (**Figure 8**).

Figure 8 – Damaged slice. The reconstruction offered (right) by superposition of the immediately previous slice emulates the anatomical region (popliteus muscle and tendon) but the area corresponding to the popliteofibular ligament had been lost during the preparation of the sample.

Specimen preparation

The desired specimen must be frozen at -80ºC (193K) at the desired anatomical position. This temperature is achieved on specialised freezers, and the anatomical parts need to be freezing for 3-5 days to achieve a uniform state. Knees IV and V were obtained from the same unfixed individual, which had been freezing at -80ºC (193K) for five days.

Once the temperature is reached, sawing process can be started. A butcher band saw will be used for this process. To avoid the rise on the temperature, it was decided to use liquid nitrogen, which maintains a temperature of -195ºC (78K), applied with a 60-

70 mm paintbrush directly on the saw and on the knee immediately before slicing and between slices. In addition, a guide stop cooled for 12+ hours at -80ºC (193K) was added between the inox guide and the saw. Liquid nitrogen will also be applied to the guide stop. Slices of 1’50 mm were obtained, with a loss of tissue between slices of 0’70 mm (saw width). For a precise measurement of the slices, the saw was set every five cuts using an electronic calliper.

Knee IV was sliced on the coronal (frontal) plane, while axial (transversal) slices were obtained from knee V. Recently sawn slices are normally covered with a layer of tissue detritus on each side. These shavings need to be removed. Otherwise they will artefact the finished preparation. Saw-dust removing was performed immediately after every slice was obtained, each of which was submerged on cold acetone (-25ºC; 248K) to avoid thawing of the slice, and brushed with small 10-20 mm paintbrushes.

Day 0	Freezing at -80ºC (193K) of fresh cadaver (not fixed)
Day 1	Slice and clean sawdust from slices Immerse slices in first -25ºC (248K) acetone bath (>90%)
Day 4	Immerse slices in second -25ºC (248K) acetone bath (100%)
Day 7	Immerse slices in third -25º (248K) acetone bath (100%)
Day 10*	Warming up of the third bath up to room temperature. Degreasing at room temperature of the slices with dichloromethane
Day 13	Impregnation in E12/E1 resin-mix (vacuum kettle) Curing preparation by sandwich technique
Day 14	Curing at 45ºC (318K) in the oven
Day 17	Open sandwich and separate resin layer (with adhered foils) from glasses).
Day 18	Sawing individual slices from the resin layer, retiring adhered foils, identification and numbering of the slices.
Day 18 to 20	Image-processing of the slices by high resolution scanning (2400 dpi)

* Slices can be preserved on the third acetone bath indefinitely. Process can be suspended at this point. Once it is resumed and degreasing starts, the rest of the procedure must follow as stated on this table.

Table 3. Summarized protocol for E12 (Biodur®) epoxy slices (<1’5mm) (Modified from Sora and Cook[58])

Clean slices must be packaged while frozen. This packages permit stacking and stabilizing the sawn tissue during dehydration, degreasing and forced impregnation. In addition, packages can be marked for a better identification and order of contained slices. BIODUR® polymer gauze and polymer grids were used (acetone and dichloromethane resistant). Gauzes are used between single slices, while grids are used on top and bottom of each package. Packages are then settled with a stabilizing grid, which can contain up to four packages of 5 to 7 slices each. In total, 23 coronal and 87 axial slices were obtained. Packages are stored in a freezer in cold acetone (-25ºC, 248K) on their first dehydration bath. The process of specimen preparation is illustrated in detail in Addendum III.

Dehydration and degreasing

Dehydration will be achieved by a freeze substitution technique. This procedure permits the substitution of “frozen” water inside the specimens by an organic solvent (acetone) to avoid the formation of crystals[62]. This substitution is achieved by submerging the slices in cold acetone (-25ºC; 248K). Using acetone instead of ethanol minimises the shrinkage of the tissue up to four times[58].

The first dehydration bath mentioned above can be developed on reused acetone, available from previous dehydration baths, as long as it has a concentration above 90%. Concentration of available acetone can be easily determined with an acetometer once a sample of it is warmed to calibration temperature (20ºC; 293K). At least once every 24 hours slices must be moved from side to side inside their bath, to remove trapped air bubbles and to avoid a double-phased solution which would compromise the dehydration of the lower slices of the tray.

After 72 hours, the slices will be transferred to a new (second) 100% cold-acetone bath. Transfer must be performed quickly to avoid drying of the slices. This procedure will be repeated after 72 hours (third bath). It is important to keep stirring the acetone every 24 hours to obtain an optimal product.

The third bath must last at least 72 hours, but slices can be preserved at this stage indefinitely. If the procedure needs to be interrupted (for instance to coordinate with other procedures) it needs to be done at this point of the technique.

Once the third bath is complete, it is brought to room temperature to start de degreasing process. Although rising the temperature could lead to an increased shrinkage ratio[40], low temperature has been reported as detrimental to final slices’ transparency, due to incomplete defatting[60]. Lipid removal is essential to achieve a defined and quite transparent slice. Slices will be transferred to a methylene chloride (dichloromethane; CH_2Cl_2) bath. This liquid is denser than acetone and slices tend to float on it. Nonreactive weights had to be used to maintain the slices submerged. This reactive works as a strong lipid remover. It is hazardous so it must be kept and handled in a ventilated hood with strict measures for personal security[63]. Personal protective equipment was used (gas-masks with carbon filters for air-suspended particles). Degreasing takes at least three days, although some studies mark a duration of one or even two[61] weeks depending on the width of the slices and their lipidic content[64].

Forced impregnation

Forced impregnation consists on exchanging the solvent the tissue is soaked in (acetone and dichloromethane) with the resin mixture that will permit plastination itself. For this procedure, BIODUR® E12 epoxy resin was used, proportionally mixed with an amine hardener (BIODUR® E1) (E12:E1; 96:26 p.b.w) (**Table 4**). Mixture must be prepared in a ventilated hood using a disposable bucket. Still in the ventilated hood, the dehydrated and degreased slices are transferred from the methylene chloride bath to the pail containing the E12/E1 mixture. Grids tend to float, so nonreactive weights need to be used to maintain the slices completely immersed.

Transparentation mixture E12, epoxy resin (96 p.b.w); E1, catalyst (26 p.b.w)
a. 96 +26 = 122 b. E12 = 96/122x100= 78’7% c. E1=26/122x100=21’3% d. For example, to prepare 1000 g of E12:E1 mixture (96:26 p.b.w.) will correspond 78’7% of 1000g (787g of E12) and 21’3% of 1000g (213g of E1)
The two mixtures used on this project: Mixture A – E12:E1 (4009:1116 g – 78’22:21’78 %) Mixture B – E12:E1 (4158:1165 g – 78’13:21’87 %)

Table 4. Procedure to calculate the components proportions to mixture (Modified from Vargas, Baptista, del Sol *et al.*[65])

In order to permit the reagents’ exchange, solvents must be extracted from the tissue. To avoid heating the preparations, what could damage them, a vacuum system is used. The bucket containing the slices completely submerged in the resin-mix is covered with a holed film and introduced inside the vacuum kettle, which will be covered and sealed with a glass port. At room temperature, dichloromethane has a boiling point (vapour pressure) of 375mmHg and acetone has it at 175mmHg[66]. Therefore, at -0’50 bar (375mmHg equals 0’5 bar) bubbles should appear, as MeCl starts to evaporate into the resin and then extracted through the pump exhaust. The objective is to low pressure down to 20-30mmHg. This process takes around seven to ten hours. In the first two hours, -0'8 bar (150mmHg) pressure is achieved gradually. At this point, mainly big bubbles will be observed, corresponding to the air from the tissue or trapped between the slices. Solvents' bubbles are smaller than air bubbles[58]. The impregnation process is mainly monitored by the observation of these bubbles. Our experience indicates that the vacuum can be stopped when pressure has been constant at 15 mmHg for at least one hour and bubbles stop appearing or when bubbling is reduced notably[64]. At this point, the kettle is returned to atmospheric pressure and the pail is brought to a wider spot for the next step of the procedure.

Curing

Last stage of preservation is curing (hardening) of the preparations. The technique we used (sandwich method) is quite messy so it is recommended to cover the work place with removable foil, and use adequate protection for hands and forearms (E12/E1 mixture irritates the skin and can lead to a weeks-lasting rash).

In this casting method, slices will be trapped between plastic foils that will shape and flatten the resin around the slice. To do so each slice is processed individually. A plastic foil (acetate) will be disposed over a bottom glass plate, and above of the former slices will be laid with a separation of at least two centimetres between them. Resin mixture from the bucket will be spooned under and over every slice, and a second plastic foil will cover the first layer. At this point, trapped air must be removed from the slices manually using a spatula. It is important to avoid trapped bubbles over the slice, as they will appear as artefacts on the final product.

The second layer of slices starts immediately over a third plastic foil, which will be covering a second glass plate. Each slice is equally covered with spooned resin and covered again with the next plastic foil. This way, the 'sandwich' is constituted bottom to top: bottom glass – plastic foil – slices (covered with resin) – plastic foil – glass plate – plastic foil – slices (covered with resin) – plastic foil – glass plate – plastic foil – … – slices (covered with resin) – plastic foil – top glass. Up to five slices layers can be disposed per sandwich stack. Once it is finished, the whole block will be wrapped in plastic foil and a weight will be put on top of it, to maintain a uniform pressure over the slices and to permit exceeding resin to slowly flow out the slices layers.

The sandwich block is kept at room temperature for 24 hours and after this time it has to be brought into the oven for 72 hours at curing temperature (45ºC; 318K)

Scanning the slices

Regardless of the kind of resin used, it tends to yellow as time goes by[67]. Once the slices were obtained, they were scanned at high resolution (2400dpi) with a scanner EPSON Perfection V800photo. This allows not only preserving the original colour of the slices, but also permits an up-close study of the images and provides the basis for other techniques such as 3D-reconstruction.

Results

For a better understanding of our findings, results will be exposed following the structures mentioned in our main objectives, this being the lateral collateral ligament (LCL), the popliteus tendon (PT), the popliteofibular ligament (PFL) and the midthird lateral capsular ligament (mLCL).

Lateral collateral ligament (fibular collateral ligament)

The LCL unites the lateral epicondyle of the femur with the lateral aspect of the fibular head, lateral and distal to its apex. Its upper insertion extends proximally and anteriorly and covers part of the lateral femoral epicondyle in a fan-like fashion. Except for its insertions, it presents a cylindrical shape.

The LCL belongs to the deep layer of the PLC's anatomy. However, our findings illustrate a strong relationship with the tendon of the long head of the biceps femoris. At its insertion, the biceps femoris' tendon branches off around the LCL (**Figure 9**) defining two different kinds of fibres, superficial to the LCL and profound to it. The deepest fibres of the tendon are inserted directly onto the superior aspect of the head of the fibula, whereas the superficial portion embraces exteriorly the LCL at its distal end, before inserting independently onto the fibula's head. This way the tendon of the long head of the biceps femoris finds its insertion on the posterior aspect of the fibula's apex and the head. The disposition of the tendon on its insertion encloses the specific site of attachment of the distal end of the lateral collateral ligament.

At its proximal portion, the LCL has a close relationship with the articular capsule and it is difficult to differentiate from it by macroscopic or microscopic dissection. Coronal section (**Figure 10**) permits the distinction of the ligament from the capsule mainly at its distal portion, but we cannot identify both structures accurately at their proximal end.

Serial axial cuts facilitate the study of the division of the biceps femoris' tendon as it approaches to its fibular attachment (**Figure 11**) and their disposition around the lateral collateral ligament.

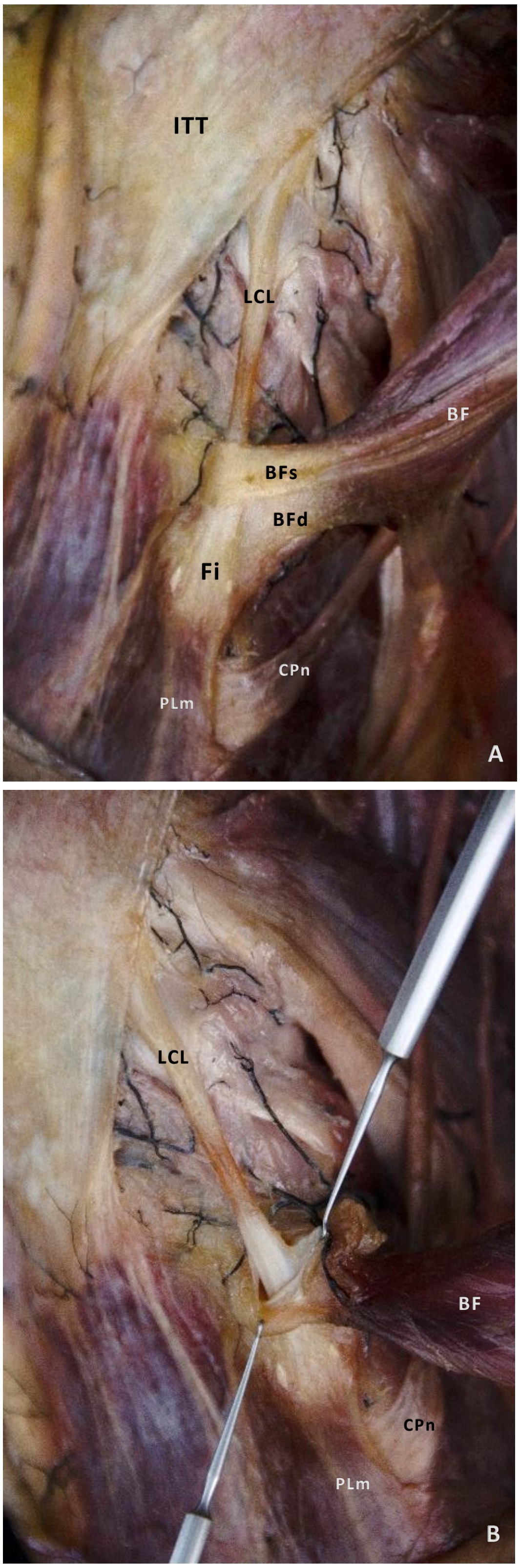


Figure 9 – Lateral view of a left knee showing the biceps femoris (BF) insertion and how it embraces the lateral collateral ligament (LCL). Superficial fibres of the tendon (BFs) cover the ligament while the deep fibres (BFd) are directly inserted onto de fibula's (Fi) head. Note how in C the BFs have been sectioned at a proximal point, and have been retired, so the dorsal aspect of the sectioned tendon is exposed. CPn: common peroneal nerve; PT: popliteus tendon; ITT: iliotibial tract; PLm: peroneus longus muscle.

Figure 10 – image corresponding to a coronal section of the PLC using E12 (Biodur®) technique. The lateral collateral ligament (LCL and black arrows) can be differentiated from the capsule (Cp) or the midthird lateral capsular ligament on its inferior half by the externalisation on the path of its fibres. Note how the capsule bends in the opposite direction to attach to the fibula (enlarged on B). Both elements appear to be touching. This might be explained by the shrinkage and the loss of adipose tissue due to the defatting process. Fe: femur; Ti: tibia; Fi: fibula.

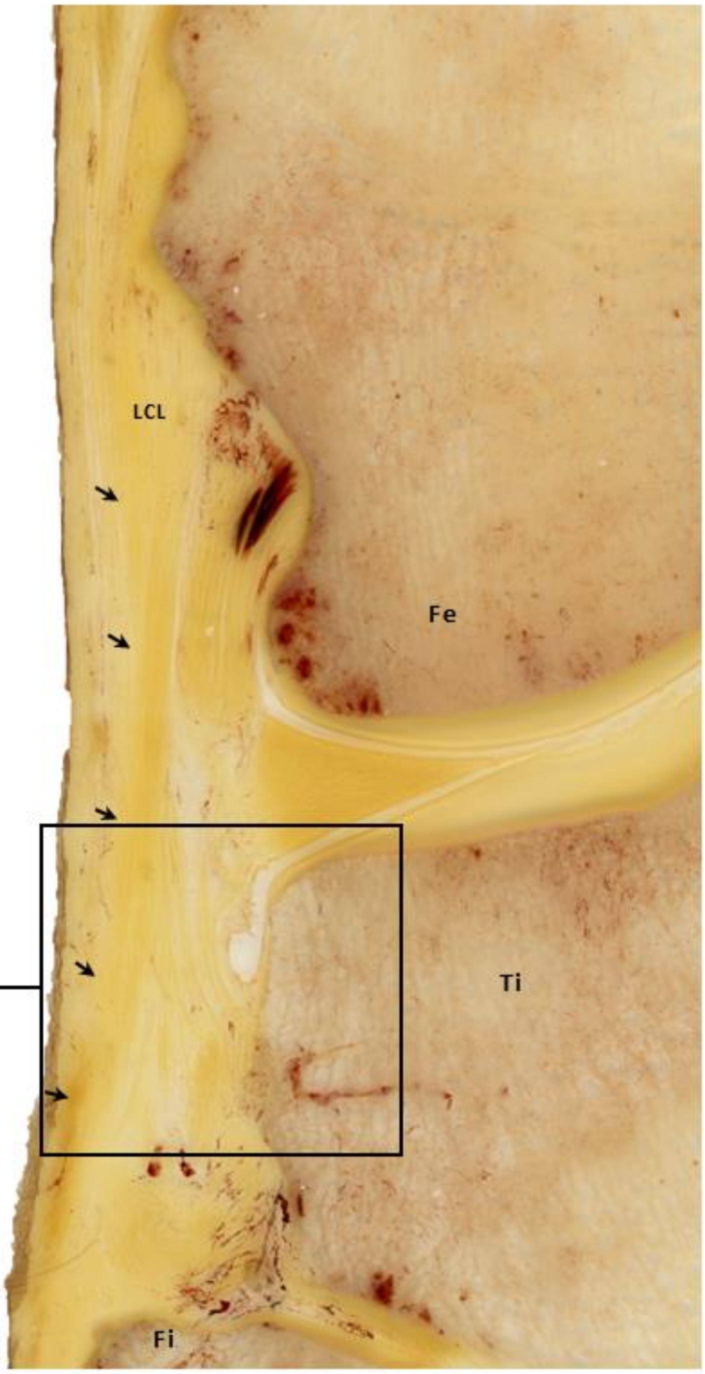
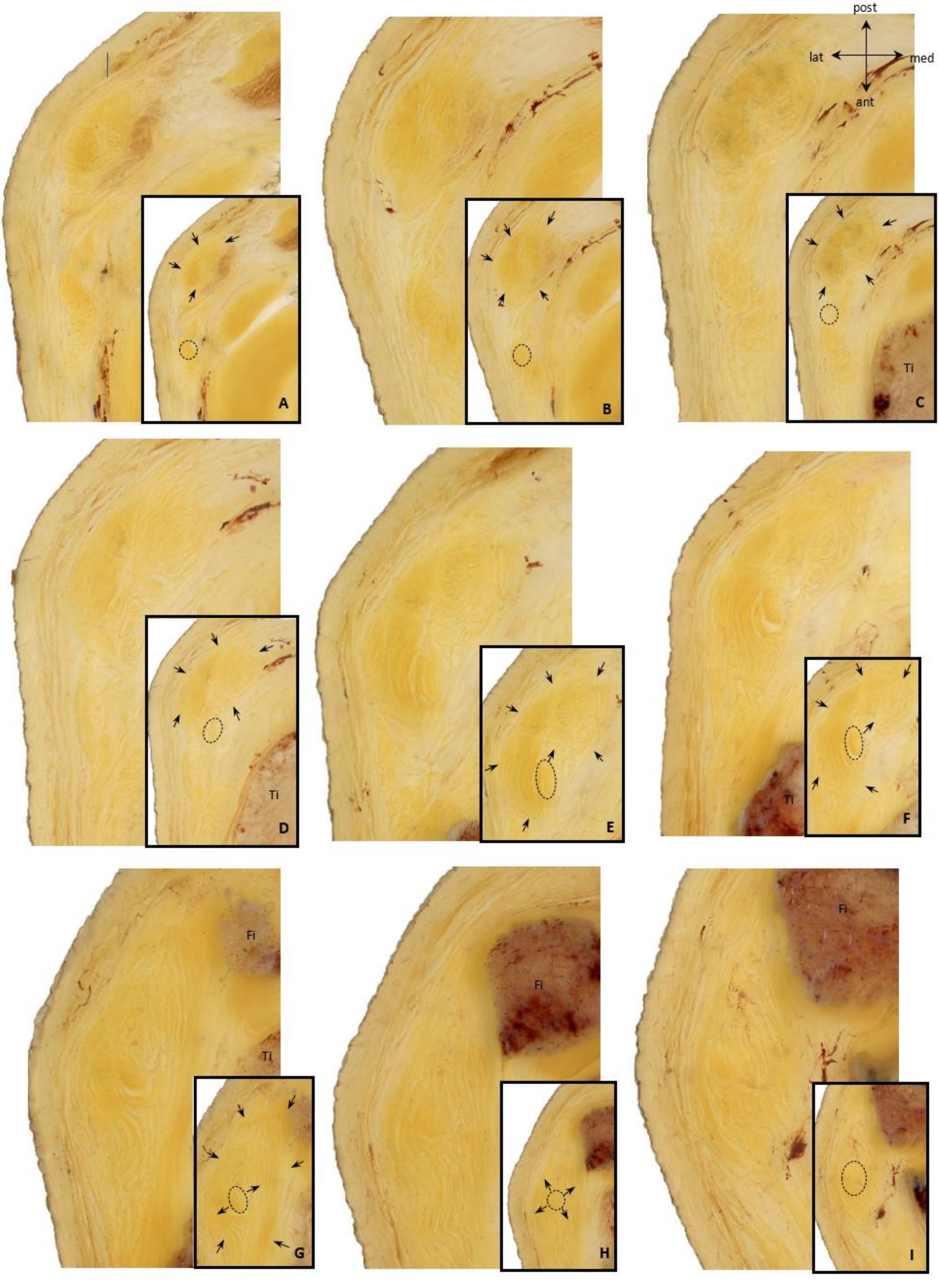


Figure 11 – (next page) Images corresponding to axial sequential sections (proximal-distal) of the PLC by E12 (Biodur®) technique. They show in detail the distal insertion of the lateral collateral ligament (LCL) and the long head of the biceps femoris' tendon (LHBF) from proximal to distal (A-I). The area occupied by the LHBF is signalled with black arrows and the path of the LCL appears circled. Note how the fibres of the LHBF separate in order to surround the LCL. The insertion is not fully seen in these axial cuts because of the obliquity of the fibres at this point. Ti: tibia; Fi: fibula.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Popliteus muscle and popliteus tendon

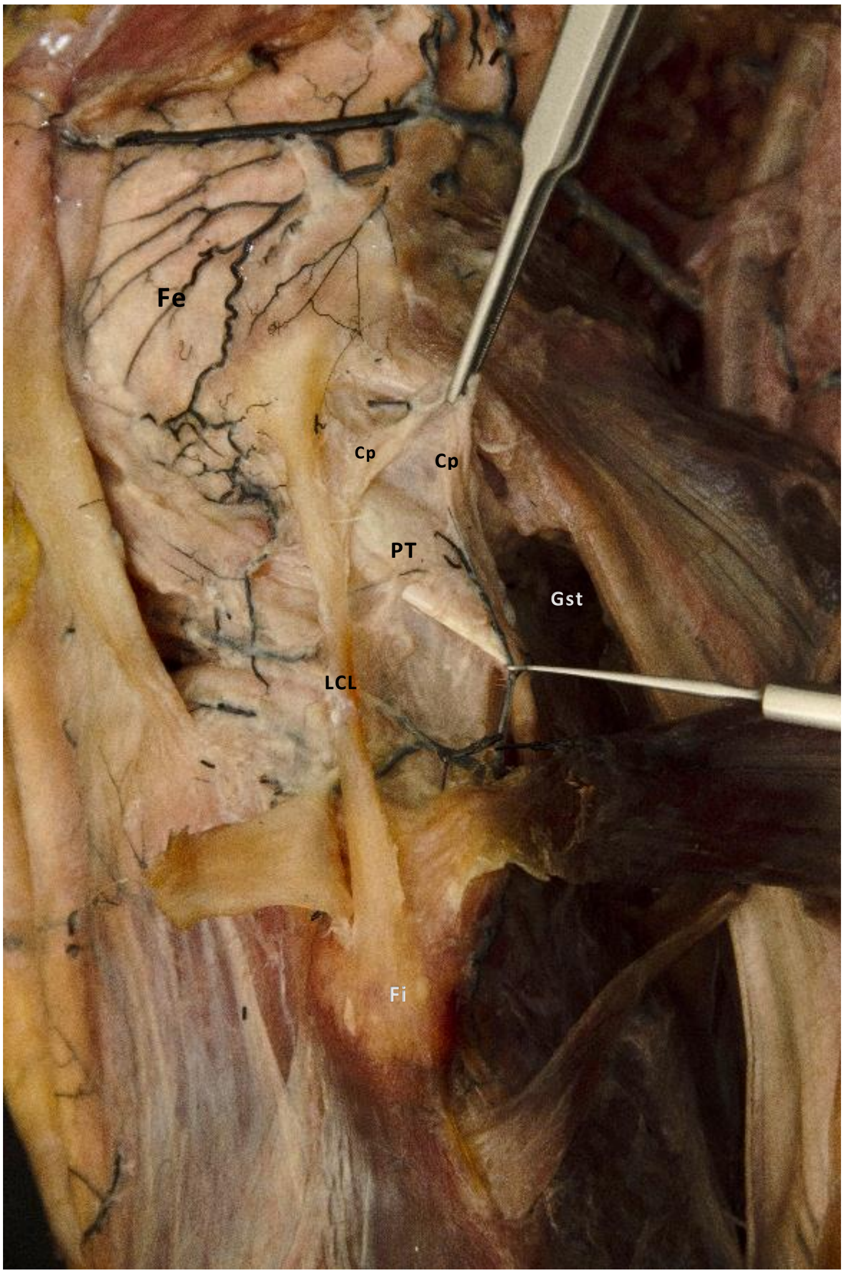


Figure 12 – lateral vision of a left knee, illustrating the popliteus tendon as seen at its entrance into the capsule, deep to the LCL. The gastrocnemius (Gst) is set aside to show the connections between the popliteus tendon (PT) and the capsule (Cp). Fe: femur; Fi: fibula.

The PM has its origin proximal to the soleal line, at the posterior aspect of the tibia. It describes a craneo-lateral path to insert onto the lateral epicondyle of the femur, anterior to the LCL. Its tendinous portion goes intra-capsular shortly after its musculotendinous junction and attaches to the femur while maintaining a narrow relationship with the capsule (or the synovial membrane, our dissection (**Figure 12**) is unable to tell apart one structure from the other). Therefore, the tendon is kept covered by a capsular reinforcement, while its deep aspect can relate to the synovial membrane.

Axial cuts of the PT at its intracapsular path show the tight relationship between the structure and the articular capsule (**Figure 13**). The most external aspect of the tendon seems to be attached to the capsule or directly covered by it, whereas its intern aspect faces the synovial space. Therefore, the PT is surrounded by connective tissue, this being compatible with the capsule itself (externally) and the synovial membrane (internally).

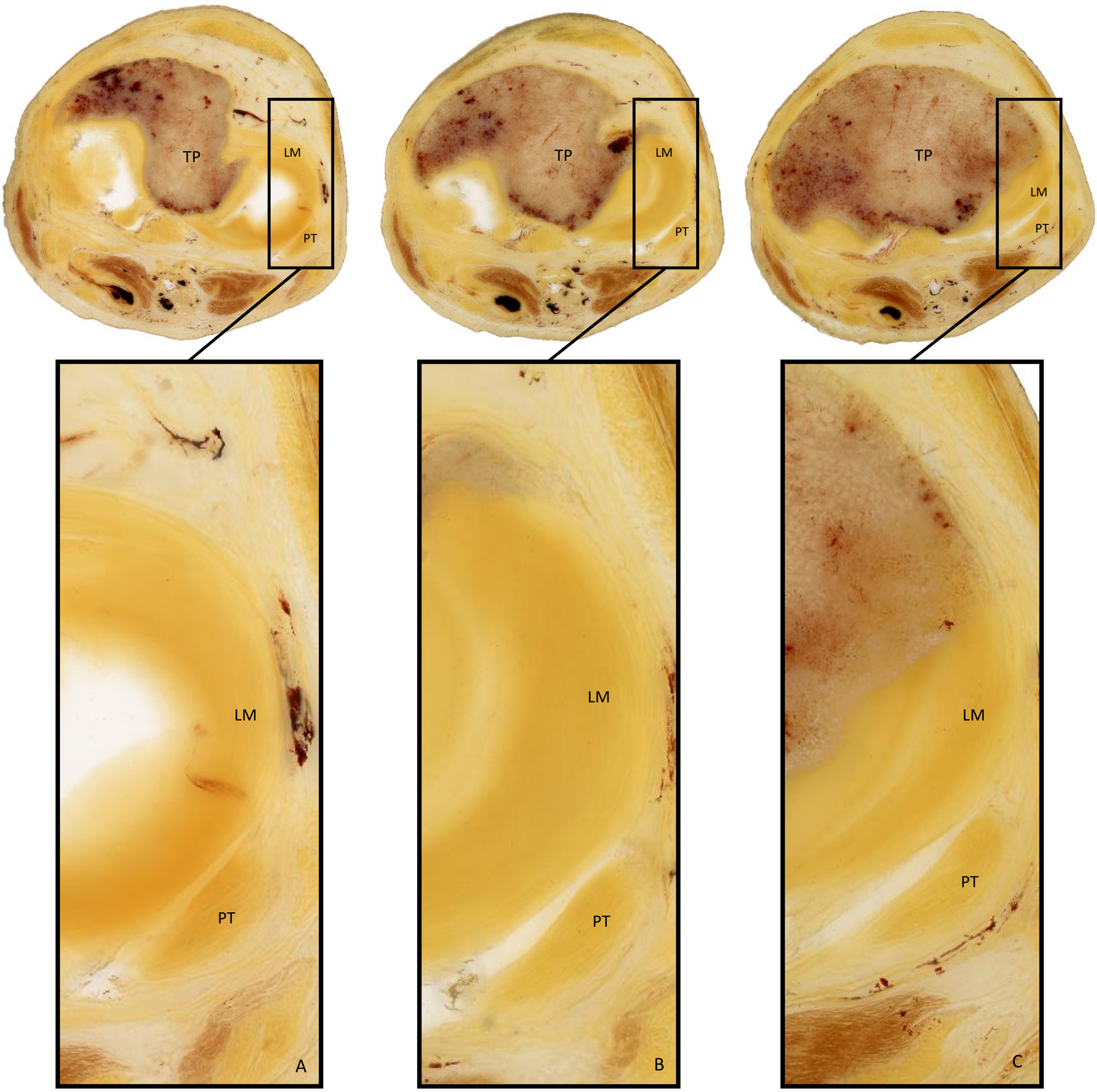


Figure 13 – serial axial cuts of a left knee disposed proximal to distal (A-C) at tibial plateau (TP) level (caudal vision). Connective tissue around the popliteus tendon (PT) is independent from the one surrounding and limiting the lateral meniscus (LM). The popliteus tendon attaches to the capsule and may be covered by synovial membrane at its inner aspect

As it is bundled up by connective tissue, the presence of tendinous fibres attaching directly to the meniscus should be dismissed. The same axial cuts evidence a complete independence of the PT from the meniscus, showing no connection between both structures and, even more, they emphasise a virtual space in-between, allegedly containing synovial fluid. Nevertheless, at some point (**Figure 13-A**) undetermined tissue can be described between both structures, it being morphologically different from both the PT and the LM.

This tissue is also reported in the coronal section, which permits identifying the presence of a structure between the PT and the LM (**Figure 14**). These might correspond to fibrous tissue or the synovial membrane itself, but image shows it shouldn't be considered part of the tendon. We could not identify any "meniscal origin" for the popliteus tendon, but we evidence the presence of loose connections between the PT and the LM presumably through capsular or synovial fibres.

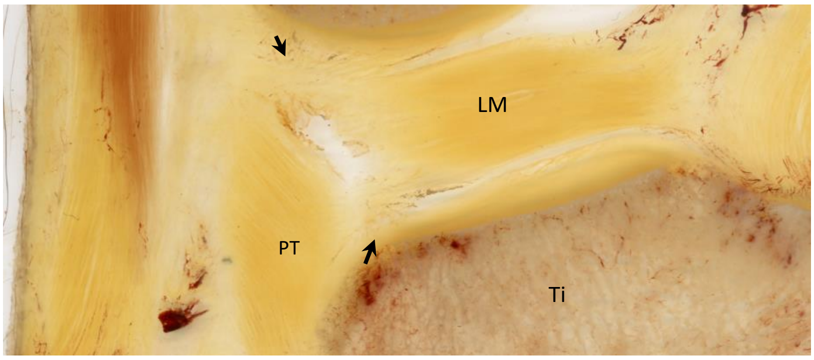
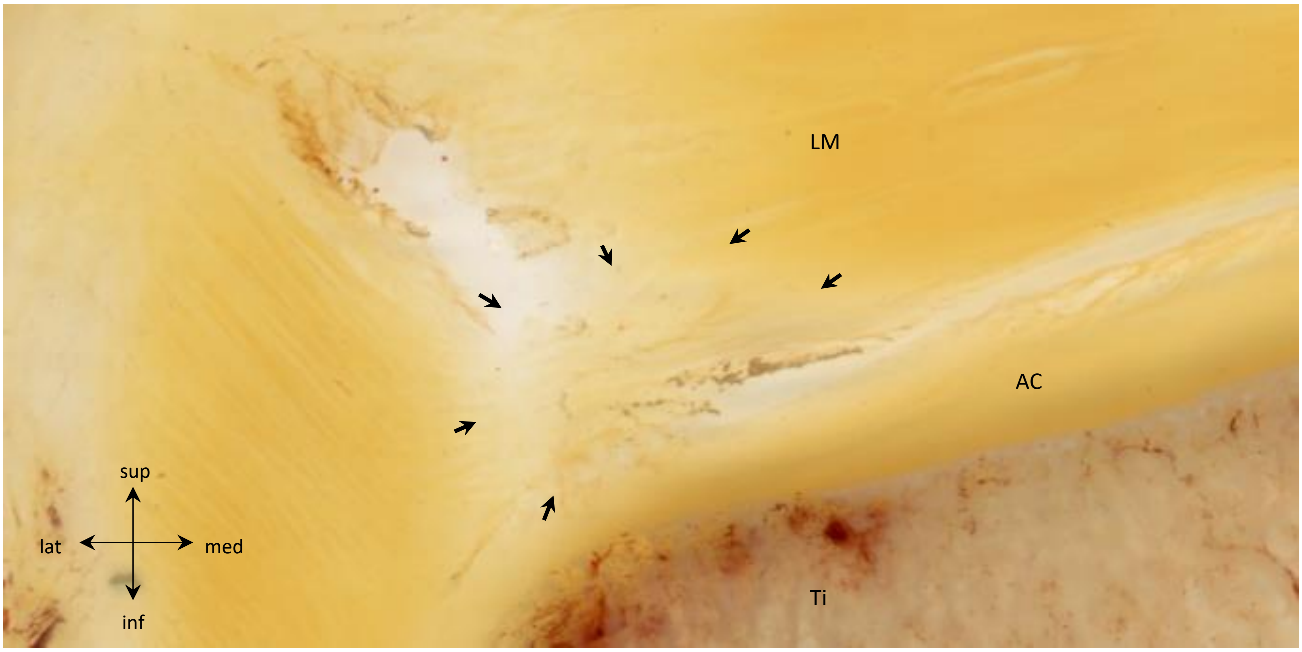


Figure 14 – coronal slice of a right knee that shows an existent relationship between the popliteus tendon (PT) and the lateral meniscus (LM). Despite not attaching directly to the meniscus, horizontal fibres of connective tissue (black arrows) relate the popliteus tendon to it. Ti: tibia; AC: articular cartilage.



Popliteofibular ligament

The popliteofibular ligament (PFL) includes those fibres that attach both on the popliteus muscle tendon and the fibular head (**Figure 15**). We have identified two fascicles shaping the popliteofibular ligament: one of them, anterior and superficial to the PT, which is narrow and even tubular-like in one of the specimens. The other one, posterior and deep to the PT, describes a trapezoid that covers a wider region of both the musculotendinous junction of the popliteus and the fibular head.

The posterior-deep fascicle is difficult to differentiate from the capsule when both structures approach the fibula's head. The observation by microsurgical stereoscope illustrates how the capsule goes under the popliteus muscle without directly attaching to it, whereas both fascicles of the popliteofibular ligament originate from the musculotendinous junction itself (**Figure 16**).

The position and disposition of the PFL hamper its study by gross dissection when we try to describe the ligament without modifying the position of its surrounding elements, as it is covered by the popliteus muscle practically in its whole. Sectional anatomy permits the unaltered anatomical study of the ligament. Detailed study of the images corresponding to anatomical sections processed with E12 (Biodur®) indicates the origin of the PFL at the musculotendinous junction of the popliteal muscle (**Figure 17**). It can be observed how its fibres have their origin along the zone of gradual transformation of the popliteus muscle to its tendon. Coronal sections, on the other hand, were useful to demonstrate the ligament's width, in addition to allowing a precise description of the insertion onto the fibula's head, at the medial aspect of the apex (**Figure 18**).

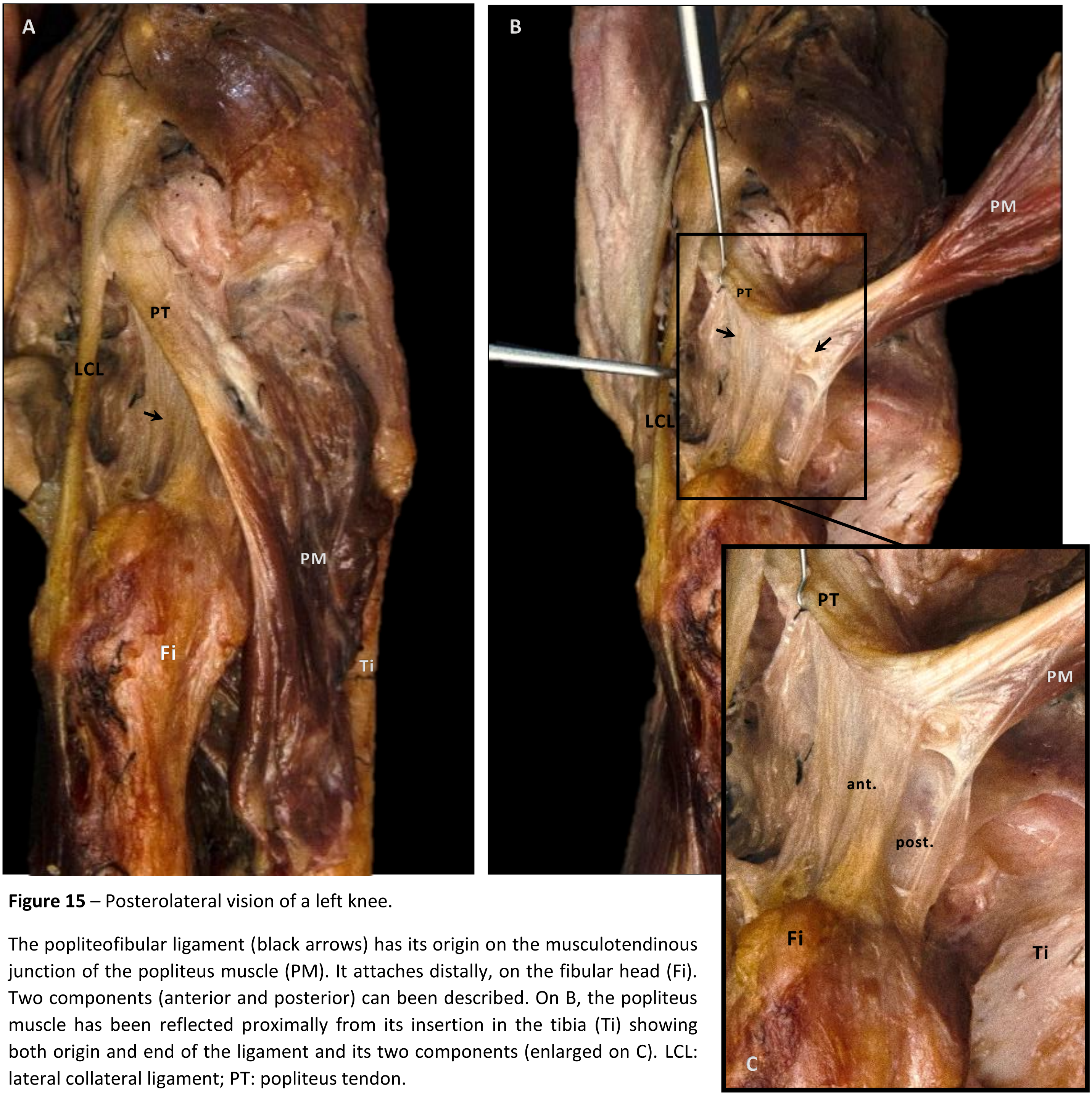


Figure 15 – Posterolateral vision of a left knee.

The popliteofibular ligament (black arrows) has its origin on the musculotendinous junction of the popliteus muscle (PM). It attaches distally, on the fibular head (Fi). Two components (anterior and posterior) can been described. On B, the popliteus muscle has been reflected proximally from its insertion in the tibia (Ti) showing both origin and end of the ligament and its two components (enlarged on C). LCL: lateral collateral ligament; PT: popliteus tendon.

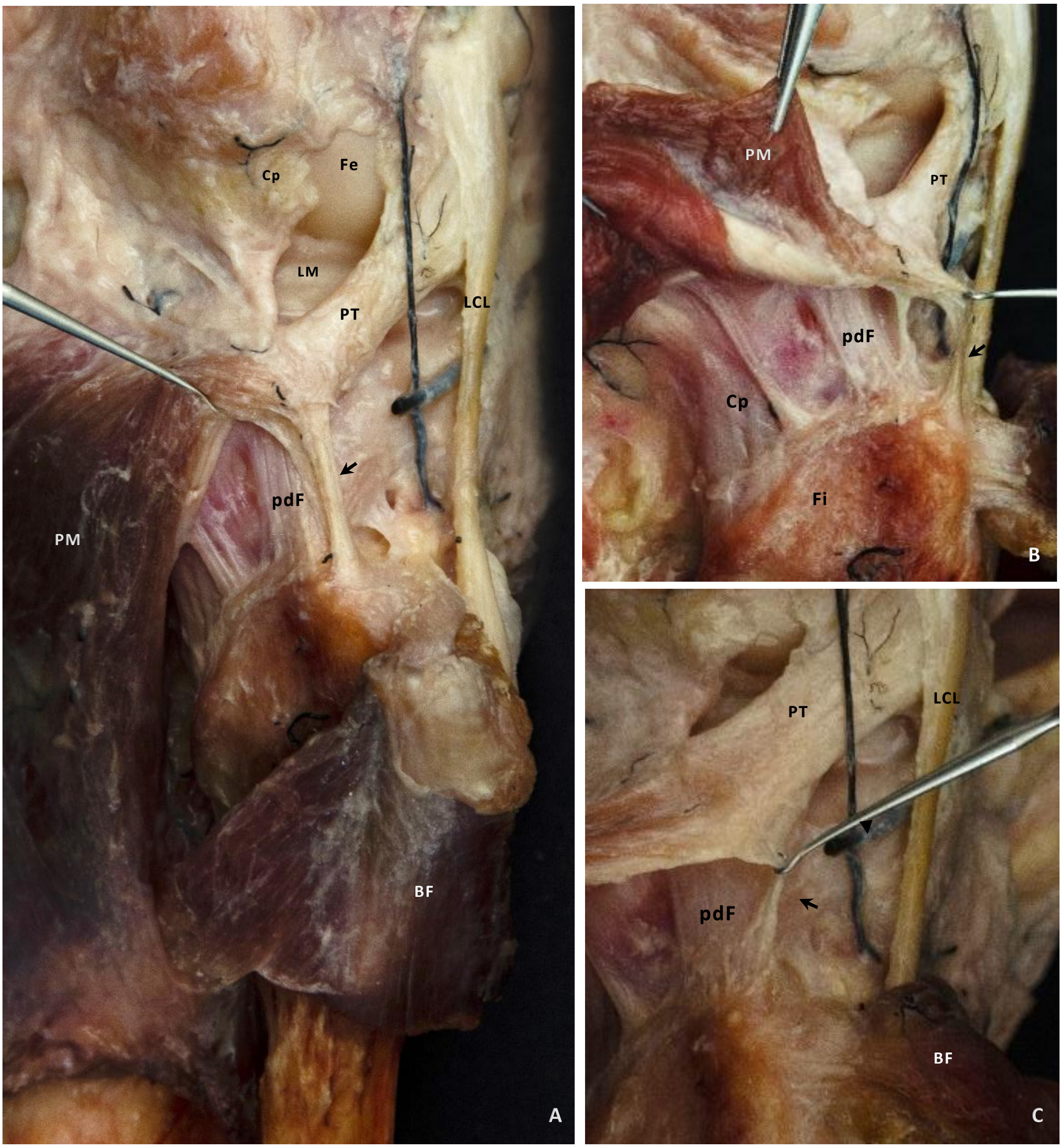
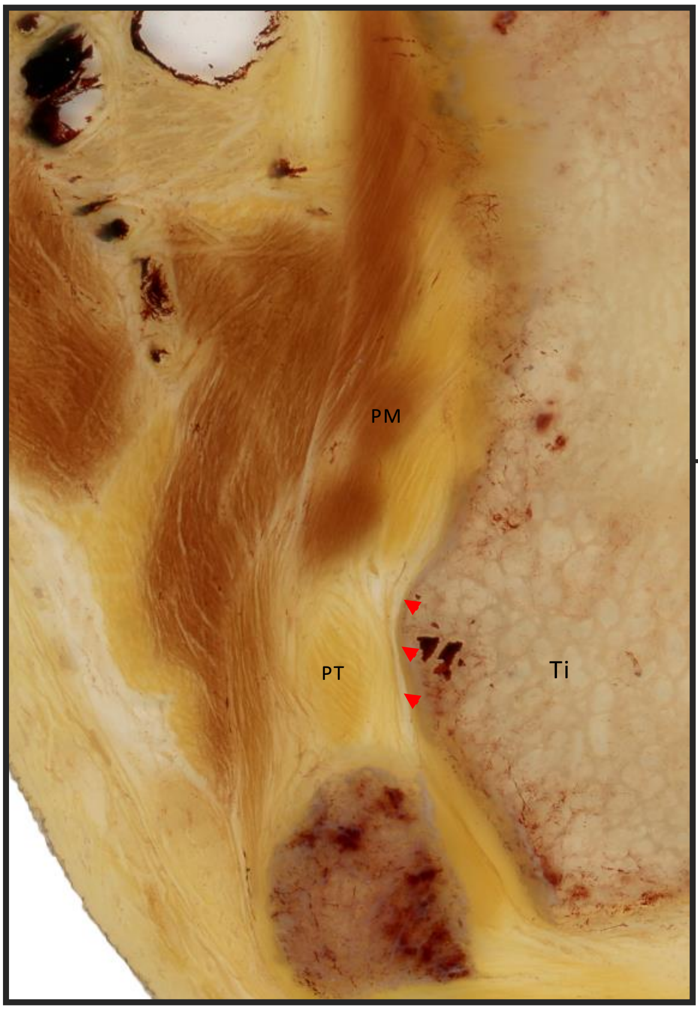


Figure 16 – posterolateral vision of a right knee. In this image part of the capsule has been dissected to show the complete pathway of the popliteus tendon (PT). The antero-superficial fascicle of the PFL (black arrow) is narrower than the postero-deep fascicle (pdF). In A, the popliteus muscle (PM) is being retracted to show both fascicles of the popliteofibular ligament and the capsular attachment onto the fibular head. Note how in B this capsular attachment (Cp) goes under the popliteus (desinserted and reflected) but does not attach to it. The capsule will cover the popliteus tendon and presumably attach to it, but it does not do so at this distal extra-capsular part of the muscle. Both in B and C the antero-superficial fascicle is retracted to show its independence from the postero-deep fascicle. LCL: lateral collateral ligament; BF: biceps femoris (sectioned and retracted); Fi: fibula; Fe: femur; LM: lateral meniscus



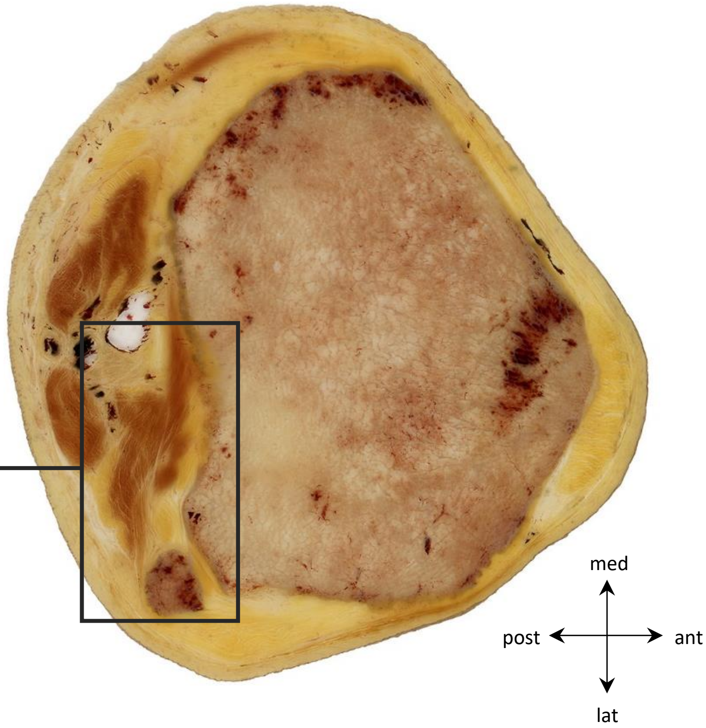
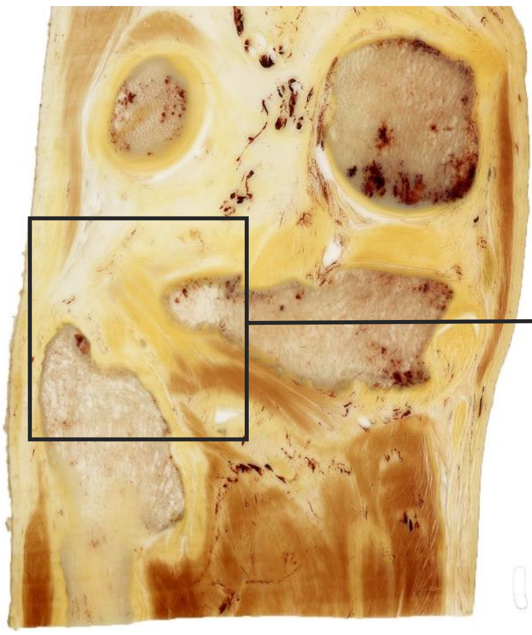


Figure 17 – image corresponding to an axial section of the PLC using E12 (Biodur®) technique, left knee, distal face. The popliteofibular ligament (red arrow heads) can be seen following the posterior aspect of the tibia (Ti) to connect the fibular head (Fi) and the popliteus. Note how there seems to be fibres of the ligament that attach to the muscular belly (PM) of the muscle whereas other fibres tend to the tendon (PT).



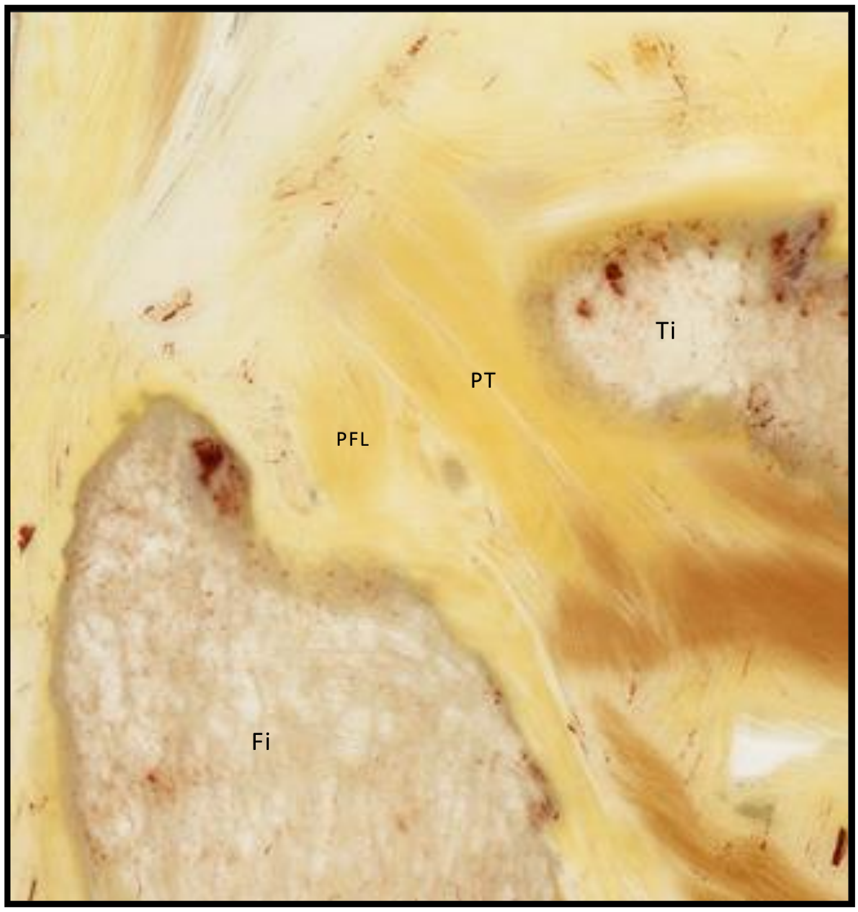


Figure 18 – image corresponding to a coronal section of the PLC using E12 (Biodur®) technique, right knee. The popliteofibular ligament (PFL) follows the same direction of the popliteus tendon (PT). Fi: fibula; Ti: tibia.

Midthird lateral capsular ligament

Microdissectional study of the lateral aspect of the articular capsule unveiled a thickening of it with a vertical disposition. Dissection technique has not allowed us to identify if it corresponds to an independent structure from the capsule. Our dissection is unable to distinguish between the capsule and the midthird lateral capsular ligament, although it evidences the relationship between the capsule (or the ligament) and the lateral meniscus at its mid-third (**Figure 19**). Study by axial E12 sheet plastinated slices presents the existence of a tight relationship between the capsule and the lateral meniscus (**Figure 20**) that we locate in a coronal posterior plane from the mLCL's, as we also observed in the macroscopic dissection.

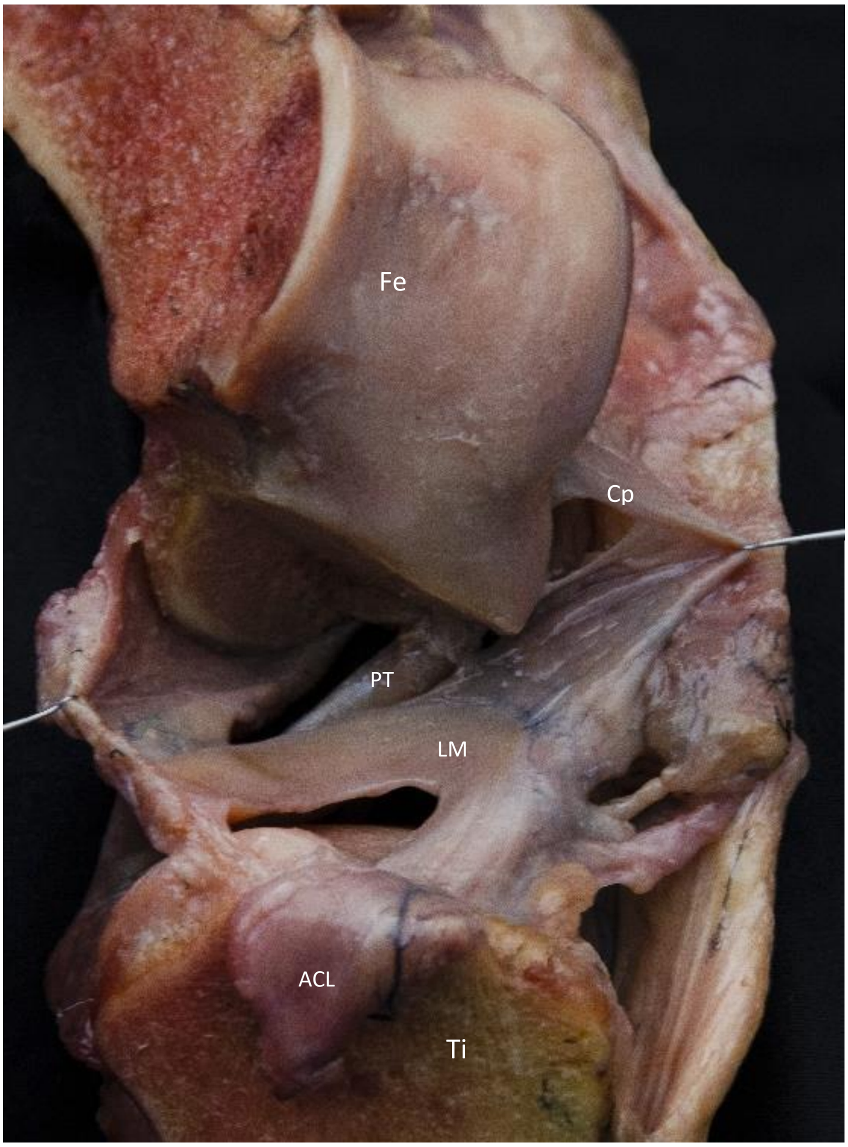


Figure 19 – gross dissection of the intraarticular aspect of the knee. Note how the whole preparation has been sectioned in a sagittal plane and only the lateral aspect is preserved. The anterior cruciate ligament (ACL) has been sectioned and moved away to permit the vision of the interior of the articulation. The anterior aspect of the lateral condyle of the femur (Fe) has also been sectioned to clear up the vision of the relationship between the meniscus (LM) and the capsule (Cp). Although we assume it is also in this portion, the mLCL cannot be described in this figure. Ti: tibia; PT: popliteus tendon

Nonetheless, a detailed study of E12 (Biodur®) plastinated coronal sections (**Figure 21**) evidenced the presence of a structure that, although related with the capsule, stays independent from it. We have observed that the mLCL follows the capsule and attaches independently to the lateral aspect of the meniscus and to the tibia, at the posterior aspect of the lateral tibial condyle. Detailed observation of the ligament following the path of the capsule leads to its femoral insertion (**Figure 22**). The mLCL reaches a recess onto the posterolateral aspect of the lateral epicondyle, which also holds the insertion of the popliteus tendon and the lateral collateral ligament. At its femoral origin, the mLCL seems to merge with the capsule and traverse the lateral aspect of the articulation independently to attach to the tibia, leaving on its wake attachments to the lateral meniscus. Nevertheless, close inspection (**Figure 22-C**) evidences a difference between the mLCL and the capsule both in colour and consistence that can be followed up to their respective insertions onto the femur. This structure, if independent of the capsule, could be defined as a deep or tibial lateral collateral ligament, considering its depth position respect to the lateral collateral ligament and the capsule. This observation could only be performed based in the coronal cuts, probably due to the disposition and narrowness of the ligament.

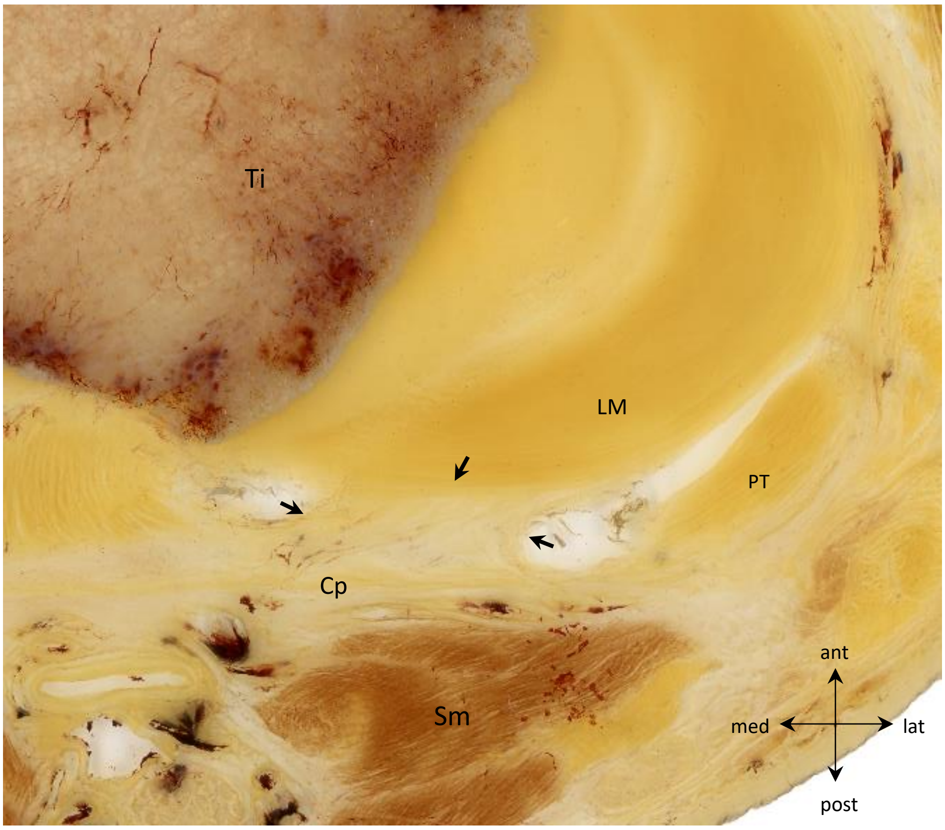


Figure 20 – this axial cut of a left knee evidences connective tissue (black arrows) between the capsule (Cp) and the lateral meniscus (LM). This image is clearly posterior to the location described for the midthird lateral capsular ligament, and so we cannot assure these fibres are compatible with it. Nevertheless, we decided to point out this adhesion site of the capsule to the posterior horn of the lateral meniscus. Connective tissue at this location could be described as an independent menisco-capsular attachment. PT: popliteus tendon; Ti: tibia; Sm: soleus muscle

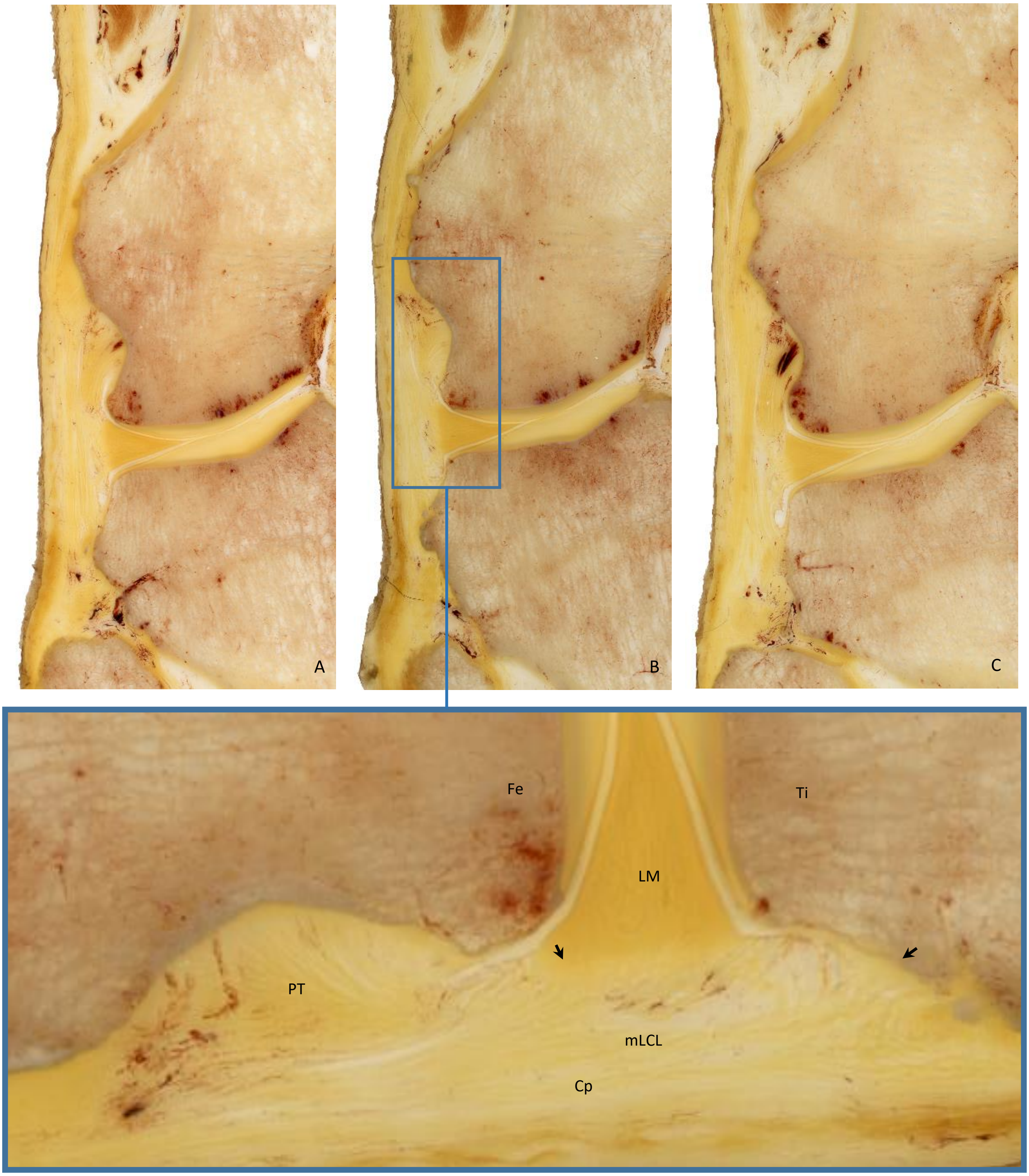


Figure 21 – serial coronal cuts disposed anterior to posterior (A-C) at the lateral meniscus (LM) level. The enlargement shows the existence of connective tissue that follows the capsule (Cp) deeply to it and attaches to the meniscus and the tibia (Ti) (attachments signalled with black arrows). Note how the popliteus tendon (PT) attaches to the femur (Fe) inferiorly to the attachments of the capsule and the fibres compatible with the midthird lateral capsular ligament (mLCL) (as it is intracapsular). Image C shows another structure, not to be confused with de mLCL or the capsule, which is the LCL, as it courses to the head of the fibula (see Figure 9 for enlargement).

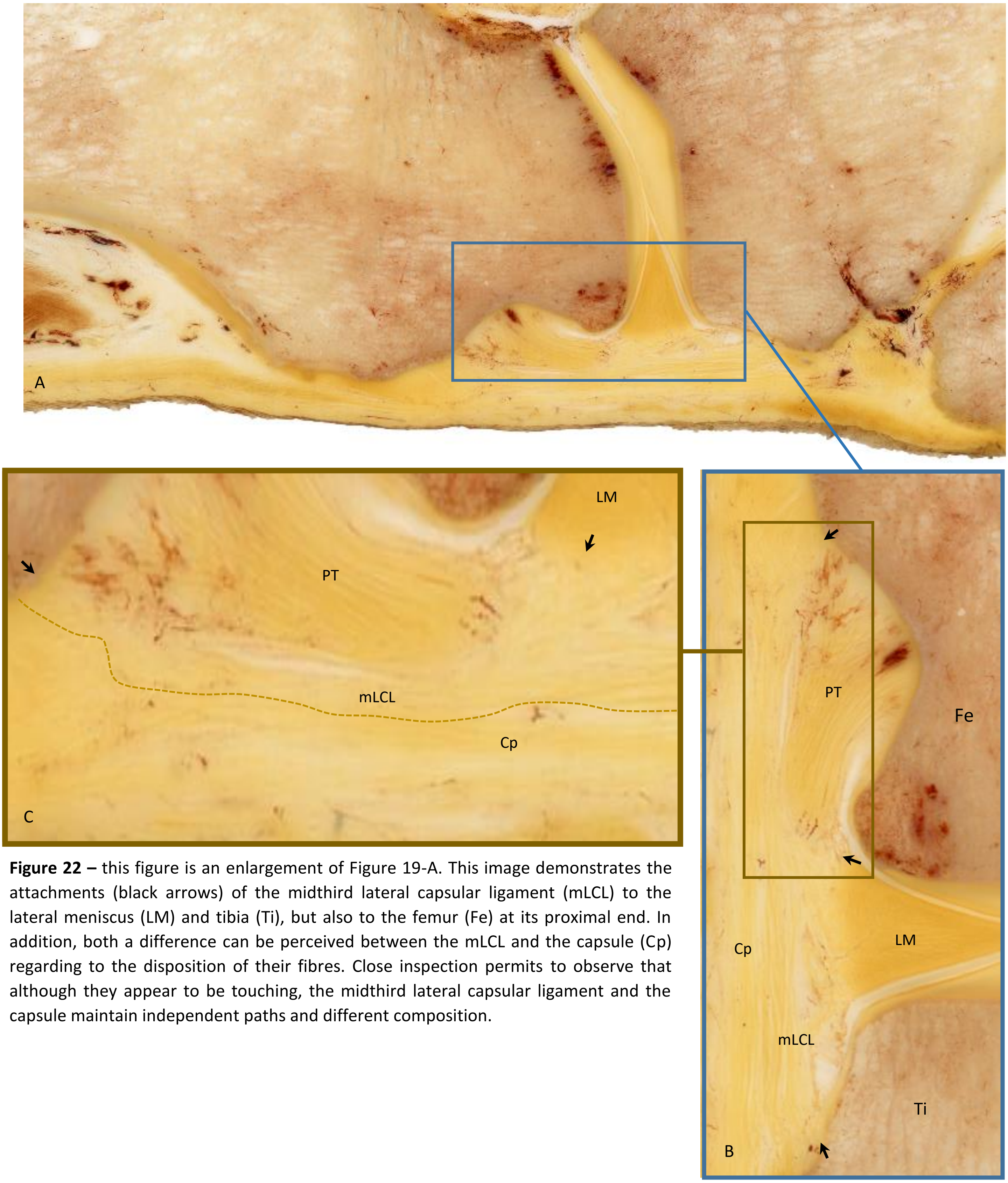


Figure 22 – this figure is an enlargement of Figure 19-A. This image demonstrates the attachments (black arrows) of the midthird lateral capsular ligament (mLCL) to the lateral meniscus (LM) and tibia (Ti), but also to the femur (Fe) at its proximal end. In addition, both a difference can be perceived between the mLCL and the capsule (Cp) regarding to the disposition of their fibres. Close inspection permits to observe that although they appear to be touching, the midthird lateral capsular ligament and the capsule maintain independent paths and different composition.

Discussion

In a 2019 editorial sent to *Annals of Joint*, Sun *et al.* considered the possibility of introducing a variation in an anatomical surgical reconstruction of the PLC of the knee[68]. They defended that their population of study would benefit of this variation, as there could be anatomical differences between Caucasian and Asian anatomy that could justify a modification of the reconstruction technique previously proposed by LaPrade *et al*[15]. In addition, they pointed out some differences between LaPrade's original anatomical description[69] and the dissections presented in an article published in the cited journal[70]. Nevertheless, the most interesting consideration of the editorial is its initial question: "*Is anatomy of the posterolateral corner* [of the knee] *always the same?"*. This is only one of the examples of the relevance of the topic, and evidences the actual need of research (and even more, anatomical investigation) that is still considerably notorious regarding the PLC of the knee. When the disagreements on the specific topics of study are analysed, the possibility of variations within the examined populations should be kept in mind, but maybe and more likely there could be a technical hitch on the techniques of the studies.

The majority of the goals proposed for this study had been already subject of other investigations. Thus, results derived from it will hardly define new statements, whereas a modification onto previous considerations could be implied. For a better understanding of the discussion, each of the sub-objectives will be assessed individually:

Lateral (fibular) collateral ligament (LCL): relationship between the LCL and the biceps femoris muscle's tendon at its distal insertion has been a matter of study due to its particular structure and disposition. In 1983 DeLee *et al*. determined that the fibular collateral ligament and the biceps femoris shared the same insertion onto the fibular head[21]. This statement confronted the previous definition of a two-portioned biceps femoris' insertion, described by Sneath in 1955[71]. DeLee's definition has been maintained and some sources are still using it for the disclosure of the distal LCL-biceps femoris' insertion[72,73]. Nonetheless, results of other investigations call into question these conclusions. Terry and Laprade avoid defining both insertions as common[74]. Shin *et al.* proposed a classification of the variants of the insertion of the biceps femoris and LCL at its distal end, even depicting a type in which the LCL would not have any direct attachment to the fibular head[75].

In the present study we did not assess all the possible variations described by Shin, but we can affirm that the relationship between the LCL and the biceps femoris tendon does not limit to forming a common insertion structure. Both of them keep independent from each other up to their insertion onto the fibula's head. The biceps femoris muscle's tendon could in fact be reinforcing the LCL's distal attachment defining a coffin that surround the distal third of the LCL.

Popliteus tendon (PT): Tria *et al.* considered inconstant the previously assumed attachment of the PT to the lateral meniscus (LM)[76]. Cohn *et al*. described fibrous tissue between the popliteus and the meniscus as two popliteomeniscal fascicles, being the anterioinferior and the posterosuperior, which define the popliteal hiatus. This structure is understood as a pathway for the PT from the tibia to its femoral attachment[77]. These attachments or fascicules could explain the indirect relationship between the popliteus tendon and the lateral meniscus, as the interconnection would not depend on fibres of the PT itself.

In order to contribute to the possible relationship with a *per se* intrasynovial structure (this being the LM), some authors have studied the PT's relationship with the capsule and the synovial space, reaching the conclusion of considering the PT as an intra-capsular but extrasynovial structure[23,78]. Nevertheless, although our dissections could not specify the tendon's relationship with the synovial membrane, E12 (Biodur®) sheet plastination technique permitted to indicate that the PT maintains an own fibrous sheath, and so it is kept apart from the lateral meniscus. This variation was studied by Kurtoglu *et al*. and presented in a paper that concluded that the PT should not always be considered extrasynovial, as the synovial membrane was reported to form a rail for the PT in some of the specimens they observed, or even to cover the tendon completely[79]. This anatomical variation could justify the differences reported in the popliteo-meniscal attachments.

Popliteofibular ligament (PFL): back in 1994 Maynard *et al*. presented a structure that, as stated on their article, had been previously omitted from literature and required to be redefined and rediscovered[17]. This was the first time the PFL was reintroduced and accepted by the scientific society, and the recent description of the ligament was stated. It recovered the original description by Higgins in 1895, considering the PFL as a division of the PT itself (fibular origin of the popliteus)[26]. Our results, based in coronal E12 (Biodur®) sheet plastinated slices (**Figure 18**), make it easy to understand why, as thickness of the PFL can be compared to the PT itself. At the same time, Watanabe *et al.* studied the structures of the posterolateral corner and specified that the popliteus had a "double origin" on the fibula's head[29], what we can nowadays understand as a first description of a double-fasciculate structure, being it compatible with the PFL. A more recent study performed by Ishigooka *et al*. which included 78 knees determined a frequent anatomical variation that permitted classifying the PFL in two types, depending on whether it was composed by a single layer (Type I) or by two layers (Type II)[30]. This second or shallow layer has been considered and studied by some authors[69,80,81] and rejected and neglected by others[24,79]. Our anatomic laminar study cannot contribute in this discussion, as the sample analysed was damaged at this point (as seen in **Figure 8**). Nevertheless, by microdissectional study of the region we could expose the existence of two differentiated fascicles. Both of them take their origin in the musculotendinous junction of the popliteus muscle. The postero-deep fascicle was observed to be thicker and presumably stronger than the antero-superficial one.

Midthird lateral capsular ligament (mLCL): or as previously named in this paper "deep lateral collateral ligament" or "tibial lateral collateral ligament" according to our results. Urban *et al.* studied the supposed constancy of two reinforcing elements of the lateral aspect of the knee: the anterolateral ligament, which would reinforce the capsule at its anterolateral corner, and the lateral meniscotibial ligament, as a constant anchoring of the meniscus to the lateral tibial condyle[82]. They concluded that the so-called anterolateral ligament was nothing else than an aponeurotic attachment of the iliotibial tract or the short head of the biceps femoris muscle, whereas there existed in fact a lateral meniscotibial ligament. This statement proposed a relevant change to the known description, as some authors had considered the meniscotibial and the meniscofemoral ligaments as part of the anterolateral ligament[28,83–85]. We have considered useful to bring forth this existing controversy about the anterolateral ligament for a reason. Vincent *et al.* made an interpretation of the anterolateral ligament (depicted in **Figure 2**) that resembles remarkably the distribution of fibres of the mLCL seen in **Figures 21 and 22** presented in our results. Taking into account that the most anterior aspect of this reinforcement has been denied as a ligament by itself by some authors[86,87], the conceptual representation could be depicting in fact the midthird lateral capsular ligament described by LaPrade[88], which could actually be considered to be composed by a menisco-tibial portion and a femoro-tibial one. The same conclusion was reached by Farhan *et al*. but the other way round, as they kept the anterolateral terminology and considered imprecise LaPrade's nomenclature[89].

Nevertheless, the tibial lateral collateral ligament should not be considered only as a thickening of the capsule, independent of which name is more accurate according to its insertions and function. A meta-analysis performed by Pomajzl *et al*. concluded that the anterolateral ligament was an individual ligamentous structure located anterolaterally in the knee after comparing 13 studies from an original poll of 342[90]. Its histological composition was studied in 2015 by Caterine *et al*. They came to the conclusion that the histological structure was compatible with ligamentous tissue, rather than fibrous-connective tissue, and so it had to be differentiated from the joint capsule[91]. They also bring back the term "lateral capsular ligament" coined originally by Campos *et al*[92].

On the other hand, different approaches have been proposed in order to simplify the region anatomy. Masferrer-Pino *et al*. include the structure in a menisco-tibio-popliteus-fibular complex, studying the anatomy focusing on the meniscus and defining its attachments and connections[31]. This complex would include the intricate system of capsular thickenings and tendinous attachments that maintain the meniscus in place while permitting its sliding mechanism, essential for the articular movement. Although it definitely is an original idea and helps understanding the function of the posterolateral structures, if separated anatomical elements can be defined they should be studied independently. However, understanding the menisco-tibio-popliteus-fibular complex as a functional association which would avoid lateral meniscal extrusion at the end of the joint's flexion is useful indeed and revisits the "popliteus complex" description by Rosas *et al*[6].

Our results stick to the concept of a structure related to the capsule but independent from it that follows continuously an intracapsular path attaching onto the femur and the tibia, and from which an attachment sprouts to the lateral meniscus.

Limitations of the study and future directions

Maybe the most important pitfall of this study is its sample value. One could inquire how could a study based only in two specimens (n=2) represent any improvement, or even change, on the current knowledge of the posterolateral corner. E12 sheet plastination is a long and expensive technique, which is not accessible for most of the clinical-anatomical research teams at a national level. This technique is protocoled on the Material and Methods section of this project. Lectors will be able to identify some differences between the protocol here presented and the one proposed by Sora and Cook[58]. Changes applied to the protocol had been previously considered and come from the experience of the research team. Shortening the times as presented on this paper does not affect the final quality of the slices and fastens considerably a *per se* long-time consuming procedure. Using only two specimens in this study will provide a new approach of the

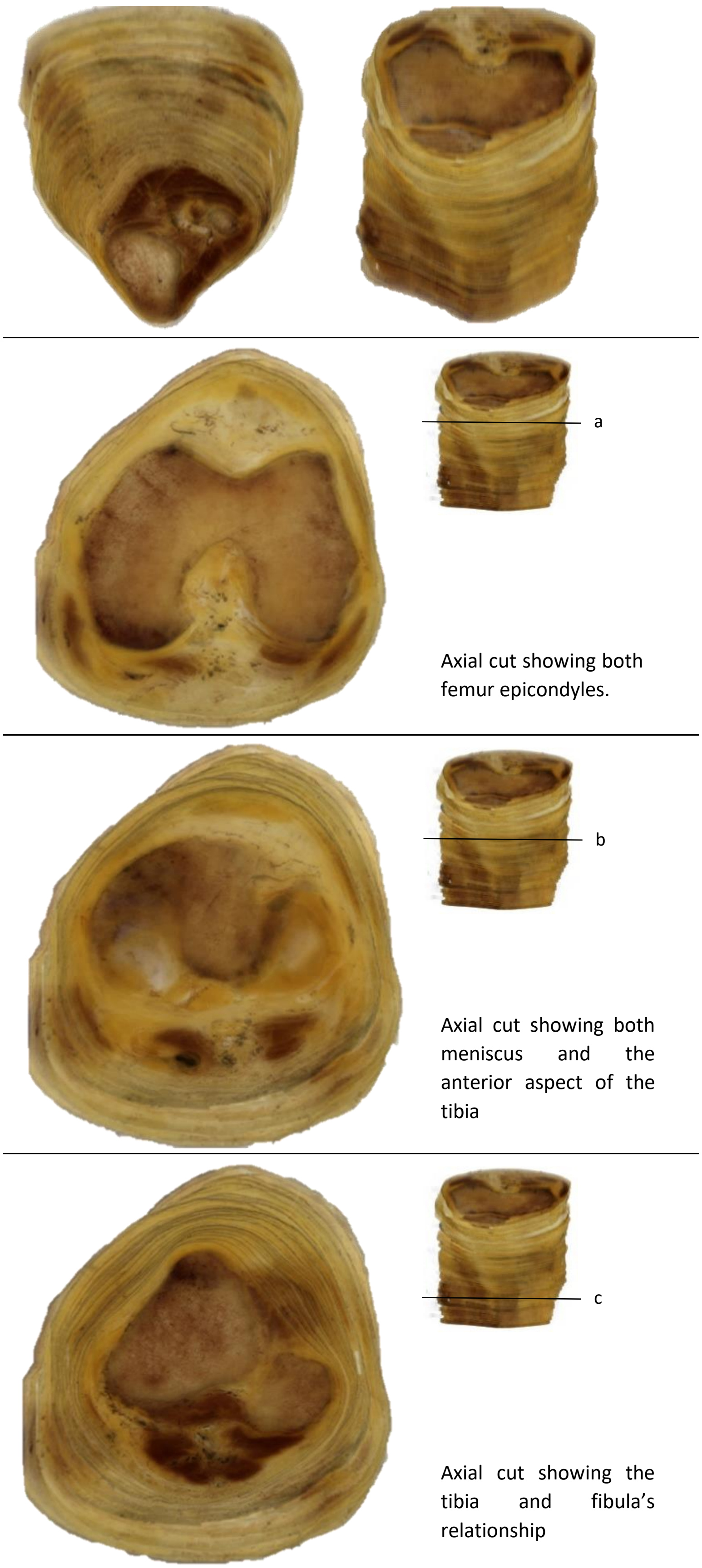


Figure 23 – 3D reconstruction permits a reliable understanding of structures using superposition of E12 slices. Sectional cuts of the reconstruction are offered at different levels (a-c) to display the possibilities of the technique. Left knee, caudal vision (CT-like).

posterolateral structures, and maybe will encourage other research teams to perform a large scale study based on this first description, but will not establish any anatomical *dogma*, and it is not intended to. This study should not be included in any meta-analysis regarding the posterolateral aspect of the knee.

Nevertheless, the sample proposed in this study is not as negligible as it seems. In 2018 Ottone *et al* published a systematic review[64] that compiled all the studies published to that date which included E12 sheet plastination on their methodologies. A comparison of the sample values is offered below (**Table 5**). E12 sheet plastination has been mainly used as an addition or as complementary investigation in the majority of projects. This fact explains the publication of experimental projects that include 1, 2 or 3 samples only on their protocol. In addition, larger samples are found in studies focused on smaller anatomical areas, unlike the anatomical region we have studied. However, the present project aims to be continued, and the sample value will be likely increased in coming revisions of the same (both micro-dissected knees and E-12 sheet-plastinated ones). Main value of this study remains on its novelty (on the area studied) and sets the bases for a wider investigation including a larger sample value.

The technique itself has its own limitations. Cuts performed “blindly” can easily damage target structures, or they can go unnoticed if the orientation of the slice does not fit the anatomical disposition of the specimen. To overcome this drawback, some authors go for performing the slices after the samples undergo an image diagnosis procedure (preferably MRI)[93–95]. Because of its high cost, it would only be recommended if additional image-based results are aimed. Another limitation of the technique (hence, of the study) is the impossibility of recovering a slice once it has been damaged. Samples can be damaged during slicing, cleaning or even during the plastination process itself. Once a slice is spoiled, it cannot be replaced by a slice from other specimens, as each preparation is sequential and has a close relation with previous and next slices. To minimise the effect a badly-performed technique could have onto the pieces, we started slicing way above the site of interest (about ten centimetres above the patella) to ensure the technique was being performed correctly once the site of interest was reached. Again, E12 plastination is an expensive technique, so it would only be recommended to expand the treated area if resulting cuts can be used in other or additional investigations.

On the other hand, gross and microscopic dissection techniques are not without limitations. While E12 sheet plastination preserved original disposition of the seen structures (two-dimensionally), cadaveric dissection implies intrinsically a distortion or disruption of local tissues. In addition, it is technician-depending and it depends on the investigator experience, so some structures can go unseen or even removed from the

Sample value (n) in existing E-12 sheet plastination studies regarding musculoskeletal tissue in humans

Johnson *et al* (2000)[96]	spinal connective tissue	n = 1	44 cross sectional slices
Nash *et al* (2005)[97]	posterior atlanto-occipital interspace	n = 13	unstated number of 2'5mm slices
Zhang and Lee (2002)[98]	cervical fascia	n = 5	unstated number of 2'5mm slices
Chen *et al* (2012)[99]	cricothyroid joint cavity	n = 4	
Liu *et al* (2013)[100]	cricoarytenoid joint cavity	n = 16	
Bernal-Mañas (2016)[93]	lateral pterygoid muscle	n = 4	unstated number of 3 mm slices
Porzionato *et al* (2005)[101]	rectourethralis muscle	n = 4	unstated number of 2-3 mm slices
Macchi *et al* (2008)[102]	longitudinal anal muscle	n = 4	unstated number of 2-3 mm slices
Al-Ali *et al* (2009)[103]	anal sphincter complex	n = 2	unstated number of 2'5 mm slices
Sebe *et al* (2005)[104]	female external urinary sphincter complex (foetal)	n = 28	ultrathin technique
Kaulhausen *et al* (2012)[105]	interspinous spacer	n = 1	unstated number of 4 mm slices
Sora and Genser-Srobl (2004)[106]	ankle syndesmosis	n = 20	20 (1'6 mm) slices per specimen
Koslowsky *et al* (2011)[107]	vascular architecture of the radial head	n = 15	unstated number of 4 mm slices (secondary slice plastination)
Koslowsky *et al* (2015)[108]	vascular supply of the proximal ulna	n = 11	unstated number of 4 mm slices (secondary slice plastination)
Fristsch (1996)[109]	connective tissue structures in the hindfoot	n = 7-14	unstated number of 3-4 mm slices (secondary slice plastination)
Sora *et al* (2007)[110]	three-dimensional reconstruction of the ankle	n = 1	ultrathin technique - unstated number of 1 mm slices
Sora *et al* (2008)[39]	posteromedial neurovascular bundle of the ankle	n = 12	unstated number of 1'5 mm slices
Sora *et al* (2012)[111]	computer aided three-dimensional reconstruction/modelling of the pelvis	n = 1	ultrathin technique – unstated number of 1'6 mm slices
Wegmann *et al* (2012)[112]	impact of posterior tibial nail malpositioning	n = 3	unstated number of 3-4 mm slices
Rath *et al* (2009)[113]	hallucal sesamoids	n = 15	unstated number of 2-5mm slices (secondary slice plastination)
Rath *et al* (2009)[114]	microvascular anatomy of metatarsal bones	n = 15	unstated number of 4 mm slices (secondary slice plastination)
Windish and Wiglein (2001)[115]	synovial sheaths in the talocrural region	n = 5	unstated number of 2 cm slices followed by 0'2mm slicing by diamond saw
Faymonville *et al* (2012)[116]	compartments of the foot	n = 12	secondary slice plastination
Oppermann *et al* (2012)[117]	microvascular anatomy of the talus	n = 6 n = 2	secondary slice plastination (3-4 mm) secondary slice plastination (2'6-3 mm)

Table 5 – Sample value (n) in existing E-12 sheet plastination studies regarding musculoskeletal tissue in humans. Complete list can be consulted on the review by Ottone *et al*[64], except from the n value, which has been looked up in every publication individually. Notes stating 'secondary slice plastination' refer to a different technique in which the slices are performed on a previously plastinated specimen.

piece by mistake if the person performing the dissection is not used to the technique. Nevertheless, serial photography of each dissection plane minimises irreversible distortion, as the dissection proceeds down to deeper levels.

In our study, lack of time has been an important drawback. Although having spent weeks in the preparation of the dissected specimens, increasing the sample of this project would (and will) require months of work.

As cited on the introduction, they are many the studies that develop three-dimensional reconstruction based on E12 laminar preparations[48,111]. The study here presented does not include 3D reconstruction on its Results, as it was not an objective of it in the first place. Nevertheless, we considered enriching for the lector or future investigators on the topic to present a very simple sample of what can be achieved by 3D reconstruction (**Figure 23**). This reconstruction has been made using Adobe Photoshop CC 2019[57], and consists simply on a superposition of all the slices, what provides a reliable reconstruction of the original sample. More precise and selective three-dimensional reconstruction can be achieved using WinSurf package from SURFdriver software©, and has been used for this purpose before[111,118,119].

Conclusions

1. E12 sheet plastination is indeed a technique with a beneficial impact and permits delving deep into the anatomical study of the posterolateral aspect of the knee.

2. The *lateral collateral ligament* (LCL) is embraced at its distal end by the tendon of the *long head of the biceps femoris muscle*, which bifurcates around the LCL without attaching to it. Both structures do not merge and they keep an independent insertion onto the fibula's head.

3. The *popliteus tendon* (PT) goes intracapsular but stays extrasynovial, what prevents it to attaching directly to the *lateral meniscus*. Nevertheless, connective tissue is reported in a transverse disposition from the synovial cover of the PT to the lateral aspect of the LM.

4. The *popliteus muscle* shows fibrous connections to the cranial aspect of the fibula's head. This *popliteofibular ligament* showed two fasciculi: a short and thick postero-inferior fascicle and a thin and long antero-superior one.

5. There exists a structure that follows the capsule at its inner aspect, and attaches to the lateral epicondyle of the femur and the lateral aspect of the tibia, with a sprouting connection to the lateral meniscus. This tissue is compatible with the *midthird lateral capsular ligament*, the *anterolateral ligament* or, as we present it, a *deep or tibial lateral collateral ligament*.

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Addenda

Addendum I	Request form for body donation to science, Faculty of Medicine, University of Girona
Addendum II	Permission and agreements with Springer Nature and Copyright Clearance Center for use of figures 1 and XX.
Addendum III	Images of the preparations of the specimens for the E12 (Biodur®) plastination process

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Que d'aquesta decisió n'informaré degudament als meus familiars, i/o amics, i/o al personal del centre sanitari en cas d'hospitalització, els quals quedaran encarregats de donar avís de la defunció als serveis funeraris de la població tan aviat com sigui possible.

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- que les despeses addicionals (certificat de defunció i altres) aniran a càrrec de la meva família .
- que una vegada utilitzat el meu cos, el mateix Servei de Donació s'encarregarà de fer els tràmits pertinents per a la incineració de les restes.
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Addendum III	Images of the preparation of the specimens for the E12 (Biodur®) plastination process

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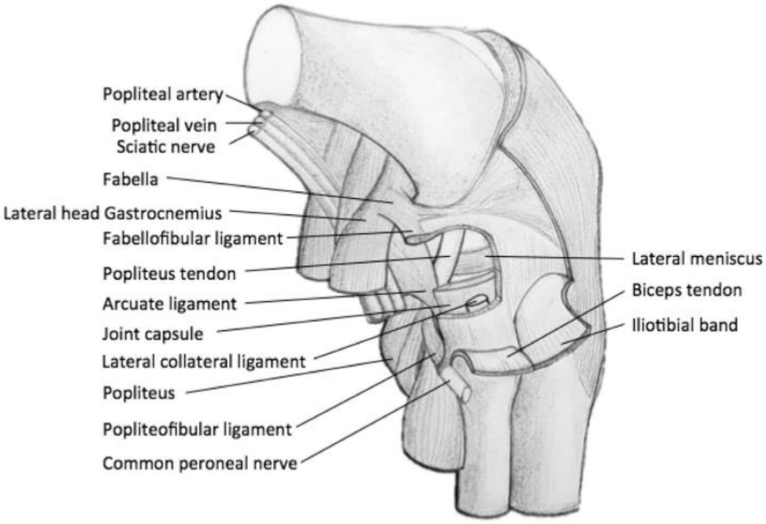

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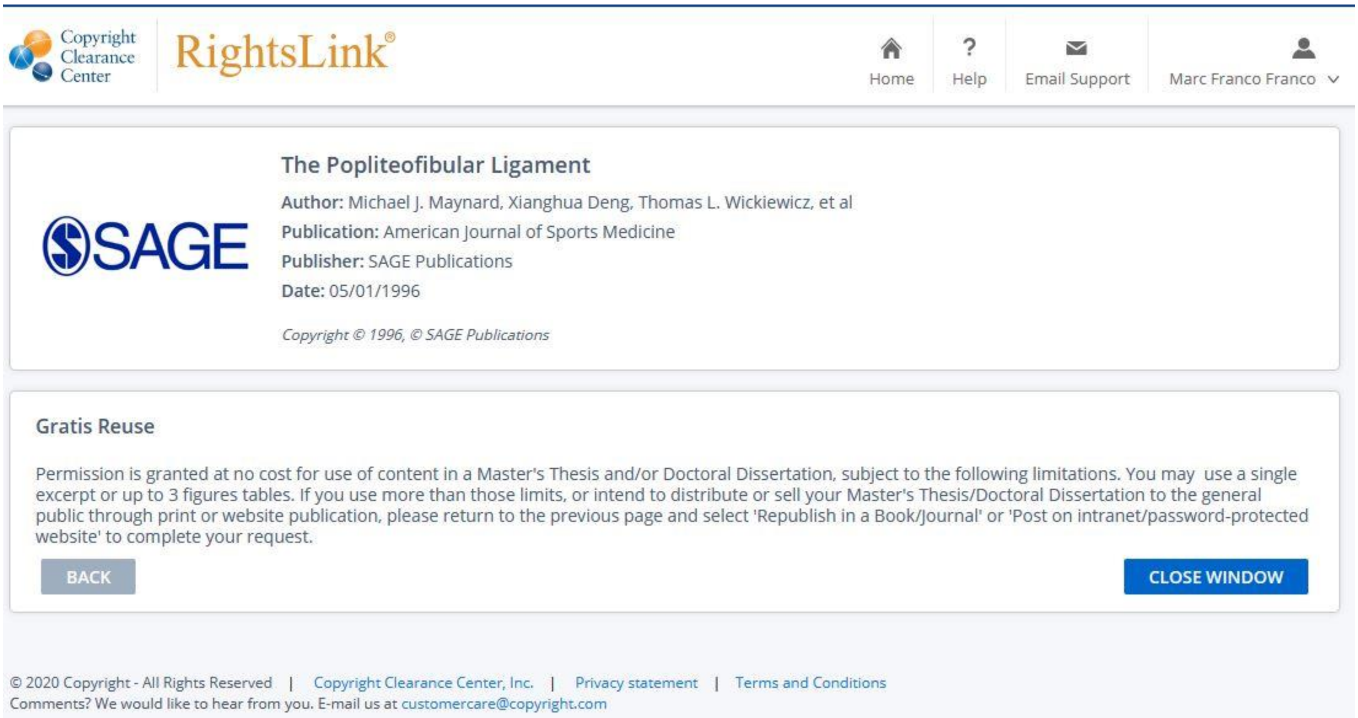

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The Popliteofibular Ligament

Author: Michael J. Maynard, Xianghua Deng, Thomas L. Wickiewicz, et al

Publication: American Journal of Sports Medicine

Publisher: SAGE Publications

Date: 05/01/1996

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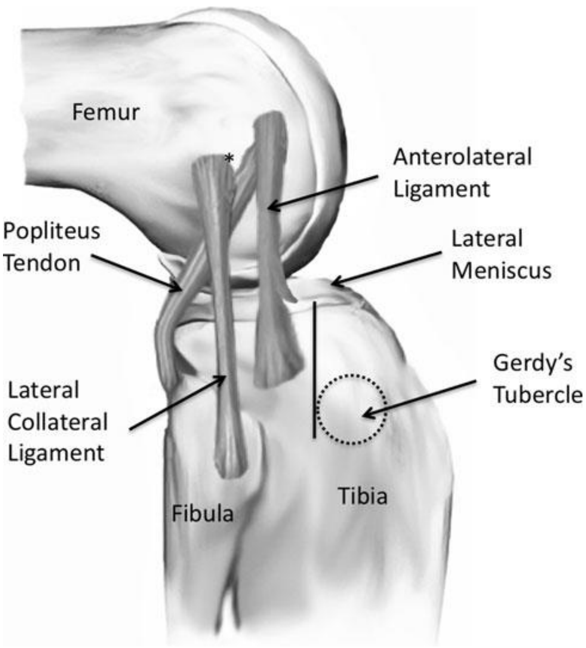

Description: this diagram presents the position of the hypothetical anterolateral ligament, understood as a thickening of the capsule. Reprinted by permission from Copyright Clearance Center RightsLink®: Springer Nature. *Knee surgery, sports traumatology, arthroscopy*. Vincent *et al*[28], 2012.

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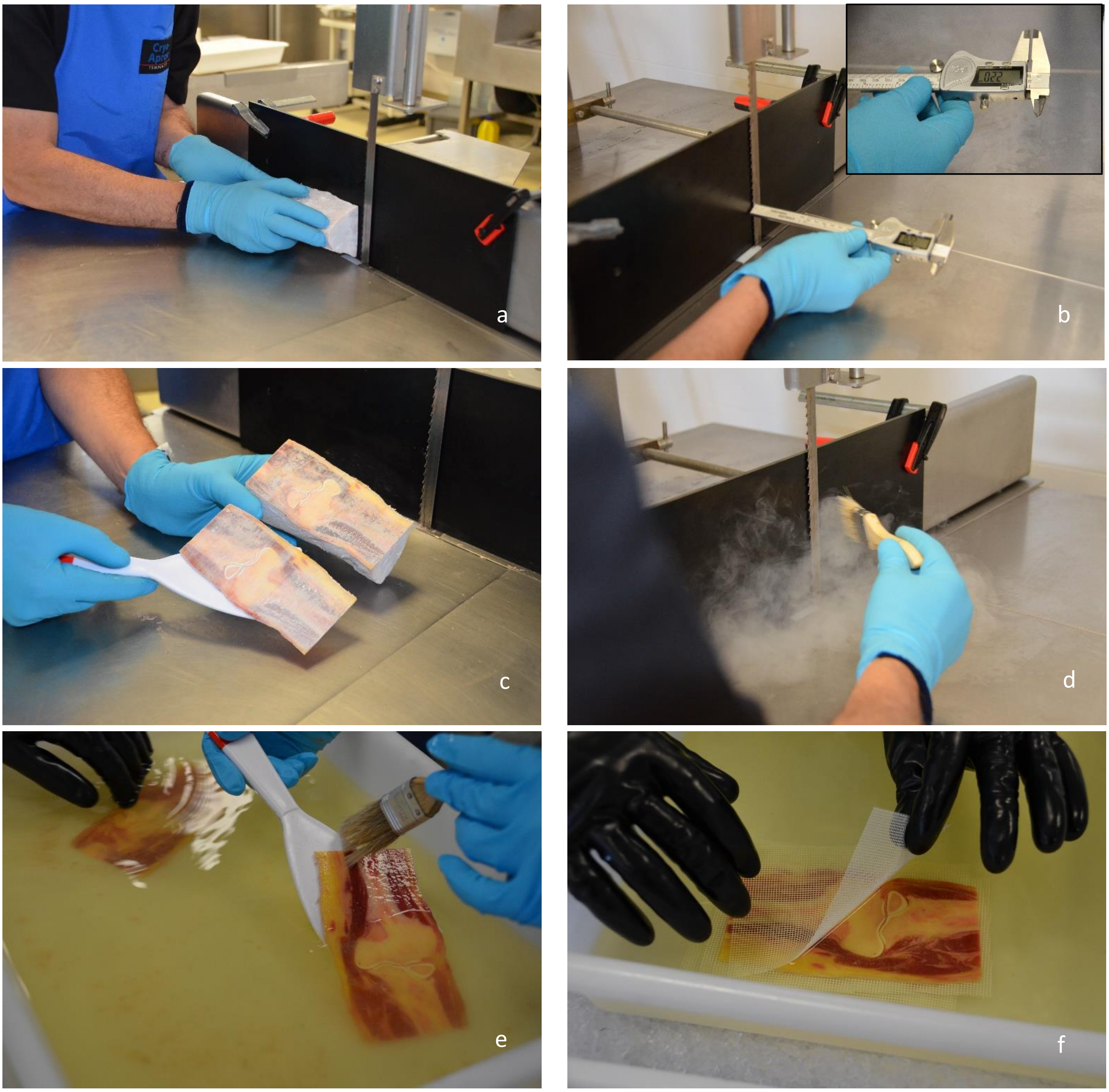

Disclaimer: all the images gathered for this addendum depict the process of E12 (Biodur®) plastination process of an elbow. The process was performed by the same team that presents this project. Due to its illustrative content and value we decided to include them as an addendum.

a – specimen frozen at -80ºC (193K) is sliced using a band saw
b – slices will be 1’5mm thin (2’20mm minus 0’7mm of saw width)
c – slices are carefully obtained from the block with the help of a spatula
d – liquid nitrogen is applied to the cutting surface between slicing to keep the temperature as low as possible
e – saw dust must be removed from the slices while immersed in a cold acetone bath at -25ºC (248K)
f – packages of up to 5 slices are made up while immersed in cold acetone. Once the packages are complete they will be submerged in an acetone bucket for the dehydration process to start.

At this point the slices will be ready to proceed to the dehydration process, followed by the degreasing, curing and scanning.