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ORIGINAL ARTICLE

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ABSTRACT

Background: The leg interosseous membrane (LIM) stabilises the tibia and the fibula. These two bones articulate at the proximal and distal tibiofibular joints. In addition, the LIM is the place of attachment of tibialis anterior muscle, extensor digitorum longus muscle, fibularis tertius muscle (anatomical variant), tibialis posterior muscle and flexor hallucis longus muscle. The specific structure of the collagen fibre network of the LIM provides durability comprising collagenous fibres that are predominately projected longitudinally, obliquely, and often transversely.

Materials and methods: 222 human foetuses (male: 120, female: 102) between 117 and 197 (median 177) days of foetal life were available for the study. The material derived from the foetal collection is stored in the Department of Human Morphology and Embryology, Division of Anatomy of the Medical University of Wroclaw.

In this study, we assessed the variability of the foetal LIM using a novel dyeing technique to identify the LIM syndesmotic structure.

Results: Overall, the study of the three types of interosseous fibres (transverse, oblique, longitudinal) of the right/left leg revealed that the fibres run in all three directions with frequencies approximating 60–70%. However, there were differences in the frequency of fibre directions and in the size of LIM between sexes.

Conclusions: After consideration of the directions and size of fibres of LIM, parts of it can be used for reconstruction of the upper limb interosseous membrane. Sexually dimorphic features of the LIM in the studied material confirm the different dynamics of lower limb growth in each sex.

Keywords: leg interosseous membrane, human foetuses, sexual dimorphism

INTRODUCTION

The leg interosseous membrane (LIM) is located between the tibia and the fibula, providing stability between these two bones. LIM also separates the leg's anterior and posterior muscle compartments [5, 8]. The tibia and fibula articulate at both the proximal and the distal tibio-fibular joints. These two joints are characterised by low mobility [13] and are important for the biomechanics and stability of the ankle joint. LIM also plays an important role in stabilising the tibio-fibular joint during lateral and medial rotation of the fibula, allowing it to resist foot twisting [21].

Additionally, the LIM serves as the attachment site for several muscles, including the tibialis anterior muscle, extensor digitorum longus muscle, fibularis tertius muscle (anatomical variant), tibialis posterior muscle, flexor hallucis longus muscle [8]. Thus, the LIM influences the ability to perform various leg movements. The specific structure of the collagen fibre network of the LIM provides durability and support. The collagenous structure of the LIM is important in various types of reconstructions following injuries or the functioning of the preserved part of the limb after amputation [10, 16].

In general, the LIM is thinner in its proximal part, while in its distal part, it is composed of several plates separated by fat. Additionally, the distal part of the LIM consists of shin binders directed diagonally from the fibula down to the tibia. Collagenous fibres are often projected longitudinally and obliquely and less often transversely. It has been speculated that the LIM is responsible for mechanoreceptive sensations in the lower limb due to the presence of mechanoreceptors: Pucini and Ruffini bodies and free neurons' endings [10].

The length and width of the LIM are sufficient to replace the antebrachial interosseous membrane (AIM). However, a potential problem arises with the orientation of the LIM fibres, which must mimic the orientation of the AIM fibres for an effective transplant. Consequently, it is not sure whether the biomechanical properties of the LIM are sufficient to perform the same function as the AIM. It is important to reconstruct the AIM when collecting the LIM [11].

There are only a few studies in the literature concerning the LIM. Merley et al. [16] evaluated its typology and structures using the microscopic methods. This has prompted the question of whether it is possible to properly stain the LIM to make the fibres that build the structure visible to the naked eye. Of course, evaluating such a novel assessing technique requires a few membranes available for dissection to be statistically reliable.

The main goal of our paper is to identify the structure and orientation of the fibres, enabling recognition of their course across the entire extent of LIM without the assistance of microscopic equipment. As a result, this paper offers a new perspective on the assessment of the variability of LIM's structure in a sample of foetal material.

MATERIALS AND METHODS

A total of 222 human foetuses (Male: 120, Female: 102) between 117 and 197 (median 177) days of foetal life were available for the study. The material came from the foetal collection stored in the Department of Human Morphology and Embryology, Division of

Anatomy of the Medical University of Wroclaw. Foetal material was obtained from local gynaecological clinics between 1960 and 1996. The foetuses were from preterm stillbirths or miscarriages. The course of delivery, as well as the decision to stop resuscitating a foetus, was made by a medical team independent of the researchers. The study protocol was approved by the Bioethics Committee of the Wrocław Medical University, Poland (resolution no 494/2020). The foetuses were stored in a prepared preservative fluid containing formaldehyde, ethyl alcohol and glycerol. Containers holding foetuses in the preserving fluid were stored in a specialised laboratory under constant temperature and no exposure to light. Methods of storing and preserving the material did not change throughout the period from acquiring the material to its use for scientific purposes [19, 20].

Foetuses with apparent anatomical and developmental abnormalities and those without basic clinical and morphological documentation were excluded from the study. Material with secondary damage and deformities due to improper storage was also excluded. The scientific value and reliability of the foetal collection have been confirmed in previous publications [12, 19, 20].

Methods

Observations for this study were collected between October 2021 and May 2022. Dissections were performed using classic anatomical techniques, which involved removing the skin and subcutaneous tissue, then gaining full access to the muscles of the lateral and posterior compartments of the lower leg, and then obtaining access to the entire interosseous membrane of the lower leg to cut and open the anterior compartment.

Another objective of the dissection was to expose significant anthropometric points for collecting metric data. Finally, the LIM was assessed: its width, length, and the orientation of its fibres were used to classify each case according to the previously created typology. The stages of the work were documented with schematic drawings and standard photographs. Photographs were taken with a digital system from Tagarno Prestige (Tagarno Innovision A/S, Denmark) and with a Sony Alfa 7II digital camera (Minato, Tokyo, Japan) stabilised on a Manfrotto tripod (Vitec Group, Richmond, UK). Measurements were taken using a Mitutoyo Absolute Digimatic digital calliper (Mitutoyo Corporation, Kanagawa, Japan). Two independent observers (KS, MS) performed Each measurement three times and the average were calculated. These averages were used for further analysis.

The staining of the interosseous membranes was conducted using a method specifically developed for this research. Interosseous membranes were covered by 1:5 dilution of the India ink and water to visualise the structure of collagen fibres. After waiting for 10 seconds for the results to appear, the membranes were washed with clean water for 12 seconds and then painted again with the India ink solution. This procedure was repeated three times. Fibre types and their orientation relative to the principal body directions were recorded for each LIM. Specifically, fibre run directions were classified as "longitudinal" (running approximately parallel to the main axes of the tibia and the fibula) (Fig.1.B), "transverse" (running from the lateral to the medial side) (Fig. 2B) and "oblique" (running from superolateral position to the infero-medial) (Fig. 3B). Based on the staining, illustrative graphics were created and compared with photographs (Fig. 1A, Fig. 2A, Fig. 3A).

RESULTS

Numbers and frequencies of different fibre run types for right and left LIM are presented in Table 1. Decidedly, long-run fibre type in the longitudinal dimension dominates both sides in nearly 95% of cases. Two other types of fibre run occur nearly at the same frequencies on both sides. These are transverse type, which occurs a little more frequently on the left side, and oblique type, which is more frequent on the right side. Wide and narrow run type fibres in width dimension are equally distributed among both sides.

Sex differences in frequencies of different run fibre types on the right and left sides are listed in Table 2. Interestingly, left but not right showed significant differences in distribution fibre run types. In male foetuses, transverse (65.0%) and longitudinal (66.7%) were most frequent, whereas in females, oblique (60.4%) type of fibre run was prevalent. These differences, though formally significant, do not indicate major quantitative differences between sexes. Short type run fibres in length dimension were observed only in male foetuses, making significant differences. Broad types of fibres were significantly more frequent in male foetuses in the right (61.1%) and left (64.9%) lower legs.

The estimation of asymmetry of fibre run types between the right and left sides is presented in Table 3. Cohen's kappa coefficient showed that in male foetuses, frequencies of types of fibres that run in length and width dimensions agree, whereas in female foetuses, they match only in width dimension. McNemar's χ^2 was not significant in all estimations.

DISCUSSION

In this study, we assessed the variability of the LIM in a foetal collection using a novel dyeing technique to identify the LIM syndesmotic structure. Our technique provided optimal visual access to the syndesmosis of the LIM. Compared to studies on the interosseous membrane of the forearm, the LIM has received considerably less attention. This is the first study where the LIM of a foetal collection has been examined. In general, there were anatomical differences in the LIM between male and female foetuses and their fibres.

Overall, the study results for the three types of interosseous fibres (transverse, oblique, longitudinal) of the right/left leg revealed that male foetuses had a significantly greater percentage of the transverse and oblique fibres in the right leg (transverse: M = 55.1, F = 49.1; Oblique: M = 53.7, F = 46.3), whereas males and females had an equal percentage of longitudinal fibres. When examining the left leg, male foetuses had a higher percentage of transverse and longitudinal fibres than females (65% and 66.7% subsequently). In contrast, female foetuses exhibited a higher percentage of oblique fibres (60.4%) than male foetuses (39.6%). Based on these results, we may surmise that their occurrence reflects development processes that are yet unclear.

It seems that the development of a collagenous transverse, oblique and longitudinal fibrous matrix of the foetal LIM in both male and female foetuses enables greater stress adaptability and elasticity of the leg — which is imperative to postnatal obligate bipedal locomotion. According to Morley et al. [16], an early process of adaptation of the lower limb structures to bipedalism is due to the present muscular structures having their attachments on the interosseous membrane. However, it is unclear whether this adaptability is due to the protein component's flexibility or to the collagen's conformation. The types of collagen fibres we described during our study of male and female foetuses and their relationship to sex seem to agree with two earlier studies [5, 13], which suggest that the arrangement of collagen fibres of the LIM has a load-bearing role with decreased flexibility.

In our study, differences in ratios between the three types of LIM fibres between male and female foetuses may also have an intimate divergent collagen distribution. This is noticeable in the anatomical size of the LIM between male and female foetuses. More male foetuses generally had a greater percentage of wide left/right LIM, whereas female foetuses had a larger percentage of narrow left/right LIM. Our study data may suggest that the higher percentage of wide LIM in male foetuses may reflect greater size in male foetuses than female foetuses, which is identifiable by the early second trimester [21]. However, this is speculative due to the need for studies in foetal LIM. No less, such a frequency distribution of LIM morphological types could be considered a dimorphic trait, forming early in ontogeny [2].

In the case of the interosseous membrane, the identification of dimorphic differences is an interesting aspect of the early development of morphological traits. Therefore, it is necessary to consider whether the variation in the structure of the interosseous membrane of the lower leg is related to the different dynamics of limb development in each sex [1]. The sex-differentiated development of some body segments is indicated, among others, by the study of Zoe A et al. [4]. In addition, the structure of LIM may be related to the course of free nerve endings that are a part of the mechanoreceptors. It remains interesting to note the presence of free nerve endings and Ruffini receptors on the outer surface in areas of potentially increased tension because of muscle work, fully developed and active during gait in later stages of life [18]. However, the question of how the course of collagen fibres in the leg's interosseous membrane develops during the prenatal period and its connection to the formation of limb mobility remains open at the current research stage.

The symmetry of anatomical structures in humans is an expression of genetically controlled development. It is evident both in the external features of the body and in the bilateral formation of internal organs such as the kidneys [14], the brain [6], or bone structures [15]. Symmetry in humans manifests in structure and organ function, e.g., symmetry of moving lower limbs during walking [9]. At the same time, asymmetries in the structure of various body segments can be observed in phylogeny and ontogeny [7]. Estimating the symmetry or asymmetry of anatomical structures can indicate "developmental stability" at different stages of ontogeny [17]. The variation of both right and left measurements and descriptive characteristics indicates the existence of fluctuating asymmetry. The assessment of asymmetry between the types of collagen fibres between the interosseous membranes of the right and left lower extremities of the foetuses indicates, through Cohen's kappa value, the possible developmental stability of the examined structures in male foetuses compared to female foetuses. This may be due to the greater eco-sensitivity of male foetuses already in early ontogeny. McNemar's $\chi 2$, on the other hand, does not confirm the existence of the phenomena of developmental asymmetry of the studied anatomical structure in both sexes.

Limitations

The study was, to some extent, selective. All foetuses were not naturally born but were miscarried or aborted for several reasons. However, we do not have any other chance to study the prenatal development of anatomical structures of the kind of collagenous interosseous membranes.

CONCLUSIONS

Determination of the degree of symmetry of the types of collagen fibre course and morphological features of the leg interosseous membranes may contribute to developing a membrane typology for interventional surgery. Differences in the dimorphic features of the intercondylar membrane in the studied material confirm the different dynamics of lower limb growth in each sex. The lack of differences among the types of interosseous membrane structure between the limbs in each sex may indicate the developmental stability of the studied foetuses, indirectly excluding disorders in prenatal development as a cause of their death.

ARTICLE INFORMATION AND DECLARATIONS

Data availability statement

The data contained in the article are available at the Department of Normal Anatomy of the Medical University of Wrocław.

Ethics statement

The study protocol was approved by the Bioethics Committee of the Wrocław Medical University, Poland (resolution no 494/2020).

Author contributions

K.S. originated idea of research paper, preparation of foetal material, film staining, soaking and methods. **A.S.** assisted in writing sections of the paper, proofreading and editing. **M.S.** prepared the foetal tissue and assisted with writing the introduction. **R.K.** assisted in writing the introduction and staining. **J.U.** worked on the interosseous diagrams. **A.P.** worked in colouring the interosseous diagrams. **P.D.** assisted in writing the discussion of the paper. **M.H.** provided intellectual assistance to the paper's ideas and with proof reading the paper. **S.K.** was responsible for tabulating the results and designing tables.
All the authors have read the manuscript and have approved of this submission.

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Conflict of interest

The authors received no funding or grants for this work from any organisation or foundation and declare that they have no conflicts of interest.

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Table 1. Distribution of frequencies of different fibre types runs in the right and left tibia.

	Right		**Left**	
Fibre run types	**n**	**%**	**n**	**%**
Transverse	49	44.1	60	54.1
Oblique	54	48.7	48	43.2
Longitudinal	8	7.2	3	2.7
	Length dimension			
Long	106	95.5	105	94.6
Short	5	4.5	6	5.4
	Width dimension			
Wide	59	53.2	57	51.4
Narrow	52	46.8	54	48.6

Table 2. Sex differences in frequencies of distribution of different fibre type runs between right and left side.

	Male		**Female**	
	n	**%**	**n**	**%**
	Right			
Transverse	27	55.1	22	44.9
Oblique	29	53.7	25	46.3
Longitudinal	4	50.0	4	50.0
	Left			
Transverse	39	65.0	21	35.0
Oblique	19	39.6	29	60.4
Longitudinal	2	66.7	1	33.3
	Length dimension right			
Long	55	51.9	51	48.6
Short	5	100.0	0	0.0
	Length dimension left			
Long	54	51.4	51	48.6
Short	6	100.0	0	0.0
	Width dimension right			

Wide	39	61.1	20	33.9
Narrow	21	40.4	31	59.6
	Width dimension			
Wide	37	64.9	20	35.1
Narrow	23	42.6	31	57.4

Table 3. Estimation of asymmetry by agreement between types of fibre run in right and left sides.

	Observed agreement	**Cohen's kappa**	**± 95% CI**	**p**	**McNemar χ^2**	**p**
	Male					
Type of fibre run	74.11%	0.01	–0.23 to 0.25	0.4638	5.48	0.1398
Length dimension	98.33%	0.90	0.71-1.0	< 0.001*	1.00	0.3173
Width dimension	86.67%	0.71	0.53–0.90	< 0.001*	0.50	0.4795
	Female					
Type of fibre run	70.59%	–0.01	–0.24 to 0.22	0.5309	2.52	0.4726
Length dimension	100%	–	–	–	–	–
Width dimension	92.16	0.84	0.68–0.99	< 0.001*	0.00	0.9999

*Statistically significantly different changes.

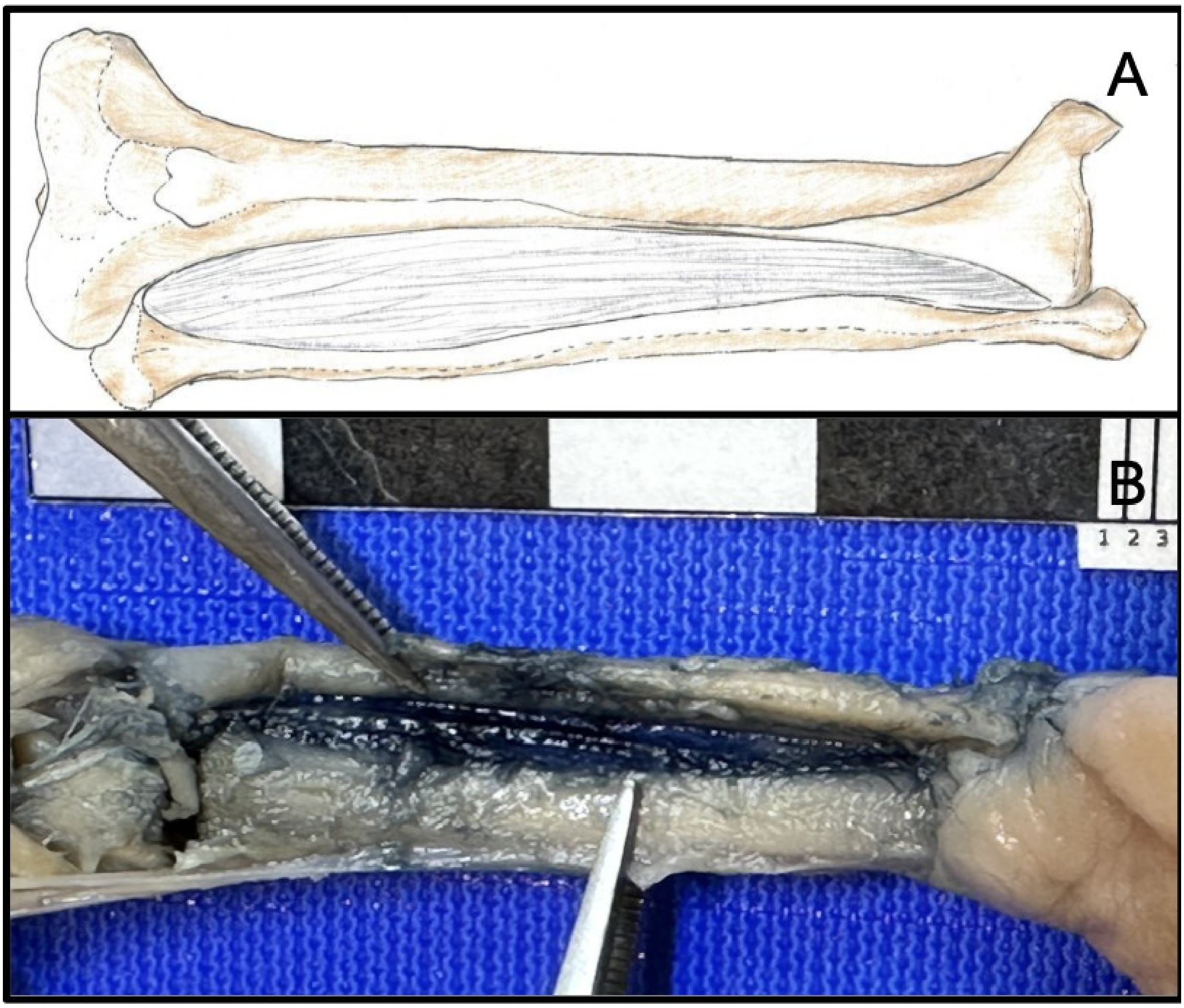

Figure 1A. Illustrative graphic of the longitudinal type designed by Jagoda Urbańska, member of the research team. **B.** Longitudinal type. In this type, the collagen fibres of the interosseous membrane of the lower leg connecting the interosseous margins of the tibia and fibula run in a longitudinal fashion.

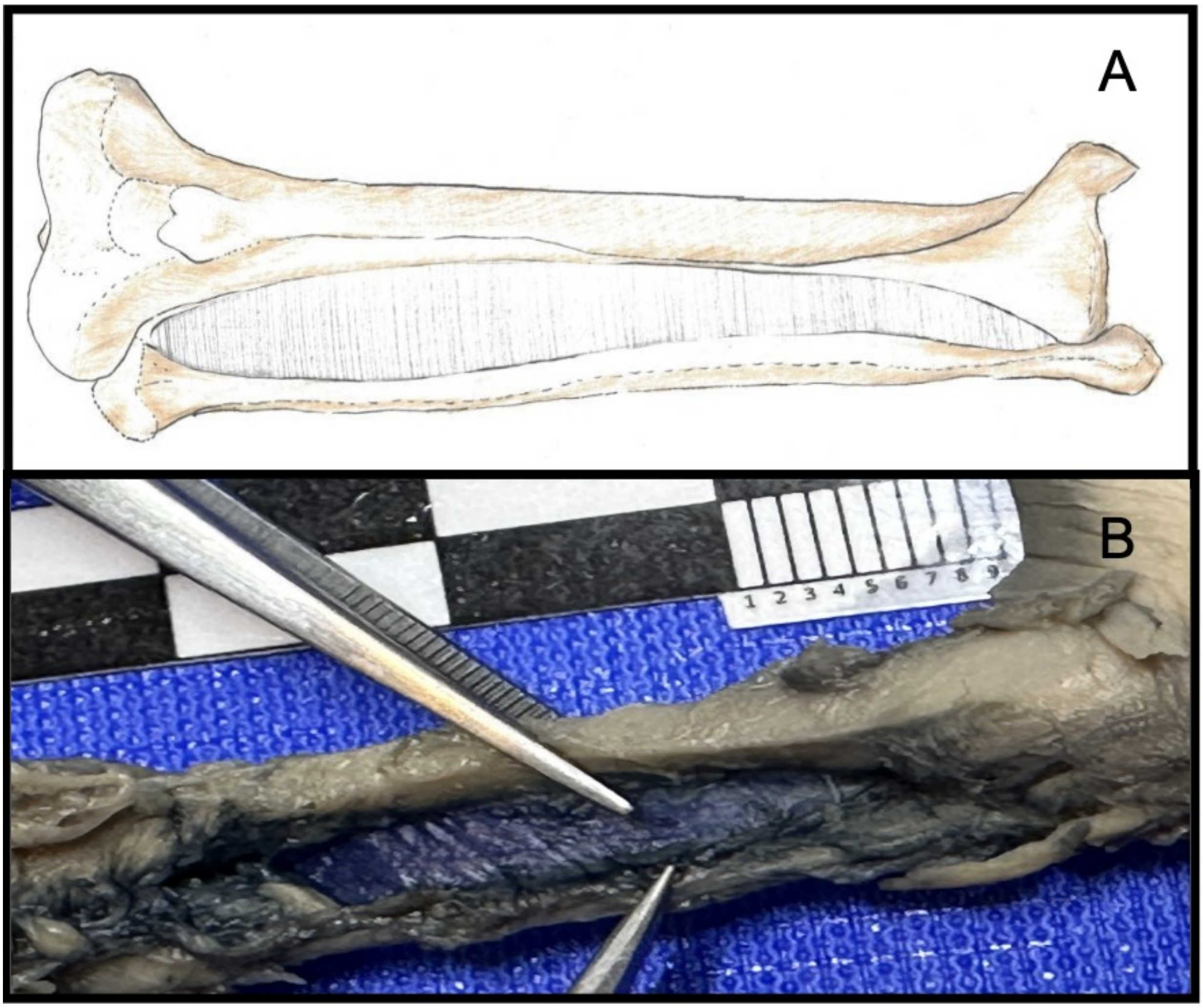

Figure 2A. Illustrative graphic of the transverse type designed by Jagoda Urbańska, member of the research team. B. The Transverse type. Collagen fibres of the interosseous membrane of the lower leg run transversely between the interosseous margins of the tibia and fibula.

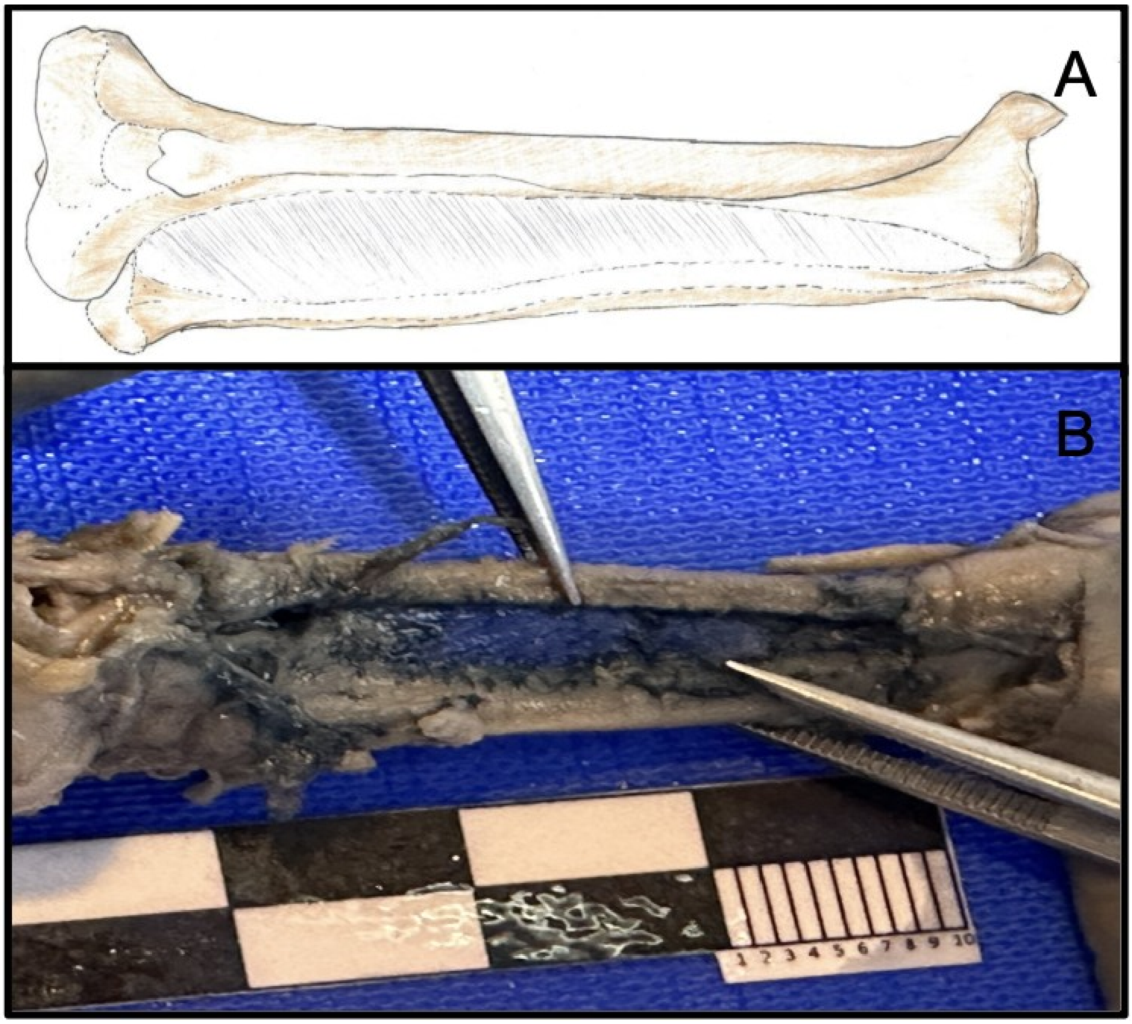

Figure 3A. Illustrative graphic of the oblique type designed by Jagoda Urbańska, member of the research team. **B.** The oblique type. Collagen fibres of the interosseous membrane of the lower leg connect the interosseous margins of the tibia and fibula obliquely.