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TITOLO TESI

**SKELETAL VARIABILITY OF THE FIBULAR ENDS AND EVOLUTIONARY
IMPLICATIONS**

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**SKELETAL VARIABILITY OF THE FIBULAR ENDS AND EVOLUTIONARY
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ABSTRACT

The human fibula, the long bone that together with the tibia is part of the leg, provides lateral support to the knee and plays an important role in ankle stabilization. This bone bears between 8-19% of the total load on the leg depending on ankle posture. The functional role of the fibula has been emphasized in studies demonstrating how foot dorsiflexion increases the degree of fibular biomechanical torsion. Yet, the morphology of fibular extremities is rarely considered in anthropology.

This thesis aims to explore and quantify the morphological variations of fibular extremities in modern humans, spanning Mid-Late Upper Paleolithic to present, and living non-human hominids, utilizing a 3D Geometric Morphometric approach.

The aims are threefold: to assess intraspecific variations of fibular epiphyseal shape, form and size in *H. sapiens*, documenting sexually dimorphic features; to explore interpopulation variability of fibular extremities from the Mid-Late Upper Paleolithic to 20th century comparing subsistence, mobility and lifestyles; and to investigate interspecific variations in fibular ends testing a possible link with locomotor and positional behavior among fibular shapes of extant hominid taxa.

Regarding intraspecific variations, relevant sex-related morphological variation in relation to fibular form and size emerged, possibly consistent with different functional requirements for the lower limb between sexes.

Regarding interpopulation variations, we found a pattern consistent with a progressive decline of activity level in conjunction with the type of terrain and shoe use, especially evident when comparing hunter-gatherers to sedentary pre- and post-industrialists, consistent with greater joint mobility, loading and range of motions in the formers compared to the latter group.

Regarding interspecific variations, we documented fibular morphological variation with functional significance among hominid taxa, possibly in relation to both phylogeny and specific loading patterns on the lower limb. We identified indicators of bipedalism in humans, and features that are shared among non-human great apes. In addition, we found features that discriminate among Asian and African apes, as well as unique morphological signals in agreement with unique positional behavior in each hominid taxa.

STRUCTURE OF THE THESIS

In **Chapter 1**, we provide an introduction on fibular anatomy and biomechanics, focusing on its epiphyseal areas. Moreover, we detail the framework of the scientific literature on leg structural properties and epiphyseal shape, focusing on intraspecific (sexual dimorphism), interpopulation (chronology, subsistence, behavior) and interspecific variability (extant hominid). Finally, following these focuses, we describe the **aims** of this work, which follow the structure of these work packages.

In **Chapter 2**, we describe the utilized samples deriving from identified osteological and museal collections, archeological collections, and online repositories. For each sample, we list the acquisition method and protocols. We also describe in detail the methodologies, software packages and statistical approaches that were employed in our 3D Geometric Morphometric (3DGM) analysis adopted in each work package. Additionally, Chapter 2 contains the **Methodology paper** that was devoted to assessing the comparability in 3DGM analysis of 3D models generated by different equipment (computed tomography and laser scanning).

In **Chapter 3**, we address the three aims of this thesis by three original research papers. **Paper I** is dedicated to the analysis of intraspecific variation of fibular epiphyseal shape, form, and size in *H. sapiens*. The paper documents sexually dimorphic features in the fibular proximal and distal extremities in three modern populations from Italy and South Africa and provides a new method for sex assessment based on fibular epiphyseal form. In **Paper II**, we explore interpopulation variability of fibular proximal and distal extremities among Italian archaeological populations dated from the Mid-Late Upper Paleolithic to 20th century with different subsistence strategies. This work details shape changes in fibular ends, comparing hunter-gatherers to sedentary pre- and post-industrialists, that are consistent with a decline of activity level in conjoint action of the type of terrain and shoe use. In **Paper III**, we offer a comparative assessment of shape variation of the proximal fibula in extant humans and great apes, using 3DGM. We quantify the variation of proximal fibular shape in each taxon and investigate a possible link with locomotor and positional behaviors. Finally, we provide the report on unpublished results of the analysis conducted for the distal fibula in extant hominid taxa, investigating the

possible link among fibular shape and locomotion repertoire.

In **Chapter 4**, we summarize our main findings and provide our conclusive remarks.



CHAPTER 1

Introduction

CONTENTS

- FIBULAR ANATOMY
 - FIBULAR BIOMECHANICS
 - BACKGROUND
 - AIMS
-

1. Introduction

1.1 Fibular anatomy

The fibula is a long, thin bone positioned laterally to the tibia: the two bones together form the leg. The fibula has a shaft and two extremities. It articulates both proximally and distally with the tibia, and with the talus distally. This bone plays an indirect role in the **knee joint**, anchoring its lateral ligaments and providing a lateral support, and has a key role in forming the lateral border of the ankle joint (White, Black & Folkens, 2011). The fibula is the smallest of the two leg bones, and, compared to all the long bones, it is the slenderest relatively to the length. The proximal extremity is placed toward the posterolateral aspect of the head of the tibia, below the level of the knee joint. Its distal extremity is slightly anteriorly inclined and projects below the tibia, laterally stabilizing the ankle joint (**Figure 1**) (Gray, 1918).

1.1.1 Fibular shaft

The shaft hosts four borders—the anterolateral, the anteromedial, the posterolateral, and the posteromedial; and four surfaces—anterior, posterior, medial, and lateral. The anterolateral border gives attachment to an intermuscular septum between the m. *extensor hallucis* and m. *extensor digitorum* on the anterior surface of the leg from the mm. *peroneus longus* and *brevis* on the lateral surface. The anteromedial border (or interosseous crest) serves for the attachment of the interosseous membrane, which separates the mm. *extensor hallucis* and *extensor digitorum* lying anteriorly from the m. *flexor hallucis longus* and m. *tibialis posterior* posteriorly. The posterolateral border gives attachment to an aponeurosis that separates the mm. *peroneus longus* and *brevis* from the m. *flexor hallucis longus* and m. *tibialis posterior* on the posterior surface. The posteromedial border (or oblique line) gives attachment to an aponeurosis separating the m. *tibialis posterior* on the medial surface from the mm. *soleus* and *flexor hallucis longus* (Gray, 1918; White, Black & Folkens, 2011).

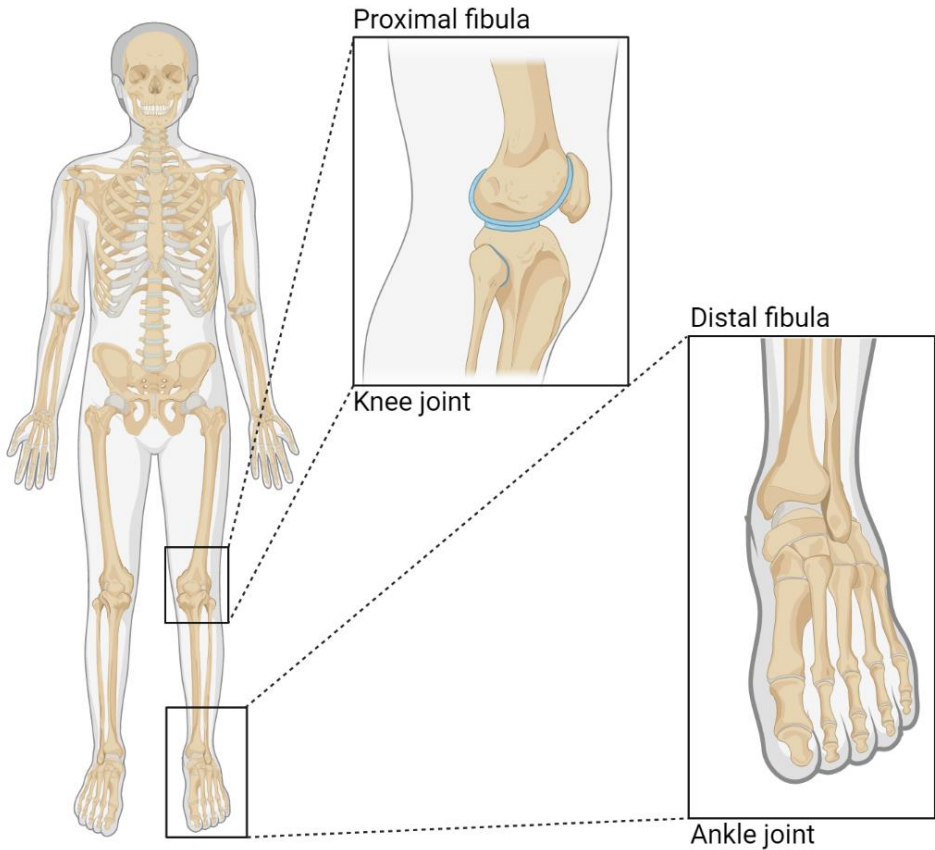


Figure 1. The fibula within the lower limb. Details of the proximal (above) and distal (below) extremities in articulation with the knee and ankle joints, respectively.

1.1.2. Fibular proximal epiphysis

The proximal fibula (**Figure 2**) in modern humans is composed of the fibular head, a mediolaterally expanded bone protrusion surmounting the fibular neck and accommodating the proximal tibiofibular articular surface (Martini, Timmons & Tallitsch, 2009). The proximal extremity of the fibular head bears the styloid process, serving as the insertion for the popliteofibular ligament

(PFL) to the popliteus tendon. At the same time, a lateral bulge holds the insertion area for the *m. biceps femoris* (knee flexor) tendon (BT) and the fibular collateral ligament (FCL) (Song *et al.*, 2018; 2020; Takahashi *et al.*, 2017). The anatomy of the proximal tibiofibular joint (PTFJ) is a plane synovial joint between the articular facet on the lateral condyle of the tibia and the facet on the head of the fibula (Sarma *et al.*, 2015). An articular capsule covers the joint, attaching just beyond the articular surfaces of the tibia and the fibula. This capsule is further reinforced by the thick anterior (APTFL) and thinner posterior (PPTFL) proximal tibiofibular ligaments, which also insert through a variable number of bundles into the fibular head anterolaterally and posteriorly (Anavian *et al.*, 2018; Scarciolla *et al.*, 2021). The APTFL inserts into an anterior protrusion in which the anterior border culminates, separating the medial from the lateral surface (White, Black & Folkens, 2011). Immediately lateral to this anterior protrusion and below the attachment of BT and FCL, the proximal origin to *m. peroneus longus* (PL), which acts to evert and plantarflex the ankle, is found (Sammarco & Mangone, 2000). Posteriorly, the PPTFL also inserts into a posterior protrusion that superiorly delineates the horse-shoe-shaped attachment area of the *m. soleus*. The anteromedial surface of the fibular head hosts the proximal insertion of the *m. extensor digitorum longus* (EDL) (White, Black & Folkens, 2011). Several studies have classified the PTFJ based on the shape and orientation of the joint surface, and significant variability has been reported (Barnett & Napier, 1952; Eichenblat & Nathan, 1983; Martin & Saller, 1959; Odgen, 1974a; 1974b). In general, the PTFJ ranges from a horizontal, planar articular surface, considered more mobile (i.e., in terms of the fibula), to more oblique articular surfaces, at varying angles with the longitudinal axis of the fibula and with different outlines and depths, which are considered less mobile.

1.1.3. Fibular distal epiphysis

The distal fibula (**Figure 2**) in modern humans has a pyramidal form and is flattened mediolaterally. Laterally, it culminates into the lateral malleolus, progressing more inferiorly than the medial malleolus. Its lateral non-articular surface is subcutaneous and forms the lateral knob of the ankle (Martini, Timmons & Tallitsch, 2009; White, Black & Folkens, 2011). On the inferior

margin of the lateral malleolus, the calcaneofibular ligament (CLF) originates from the anterior part (Golano *et al.*, 2010). It is anatomically positioned just below the lower bands of the anterior talofibular ligaments (ATaFL), which originates just anteriorly to the CLF, on the anterior margin of the lateral malleolus (Golanó *et al.*, 2010; Burks & Morgan, 1994). Immediately superolaterally, the subcutaneous triangular surface (STS) is above the lateral malleolus. This surface is lined by two vertical sides of the triangle that ultimately converge to become the anterior border at the apex of the triangle. Posteriorly and continuous with the STS on the lateral side of the body, the fibular groove (or retromalleolar groove) (FG) marks the posterior surface of the distal fibula and hosts the tendon of the m. *peroneus longus* and *brevis*, which plantarflex and evert the foot at the ankle, originating in the fibular head and shaft and inserting at the bases of the first (longus) and fifth (brevis) metatarsals (White, Black & Folkens, 2011). The fibular groove extends from the distal apex of the lateral malleolus proximally to the retromalleolar tubercle, the medial eminence of the posterior surface of the distal fibula. The digital fossa is medial to the retromalleolar groove (Cozzetto *et al.*, 2014). The medial surface hosts the fibulotalar proximal and distal articular surfaces (PAF and DAF, respectively) (Marchi *et al.*, 2019; 2022). The PAF is almost parallel to the sagittal plane (i.e., medially facing), with a cranial border perpendicular to the longitudinal axis of the fibula; the DAF has a subtriangular shape with a pointed apex that is variably slightly facing inferiorly (Marchi *et al.*, 2019; 2022). These articular facets, bordered superiorly by the ankle capsular ligaments, face the lateral facets of the talus and together with the tibia form the talocrural joint. Behind and beneath the DAF is a rough depression called malleolar fossa, which gives attachment to the lateral insertion of the posterior tibiofibular and the posterior talofibular ligaments (Ebraheim *et al.*, 2006; Golanó *et al.*, 2010; Hermans *et al.*, 2010; Perrich *et al.*, 2009). Above the fibulotalar articular facets, lies the attachment area of the interosseous membrane (ILA). This roughened, raised triangular area is part of the tibiofibular syndesmosis, and is shaped as a convex triangle. The base of the fibular triangle is formed by the anterior tubercle (also known as the Wagstaffe–Le Fort tubercle) and the almost negligible, posterior tubercle,

respectively hosting the anterior and posterior tibiofibular ligament (ATIFL and PTIFL) attachments, respectively anteriorly and posteriorly (Hermans *et al.*, 2010).

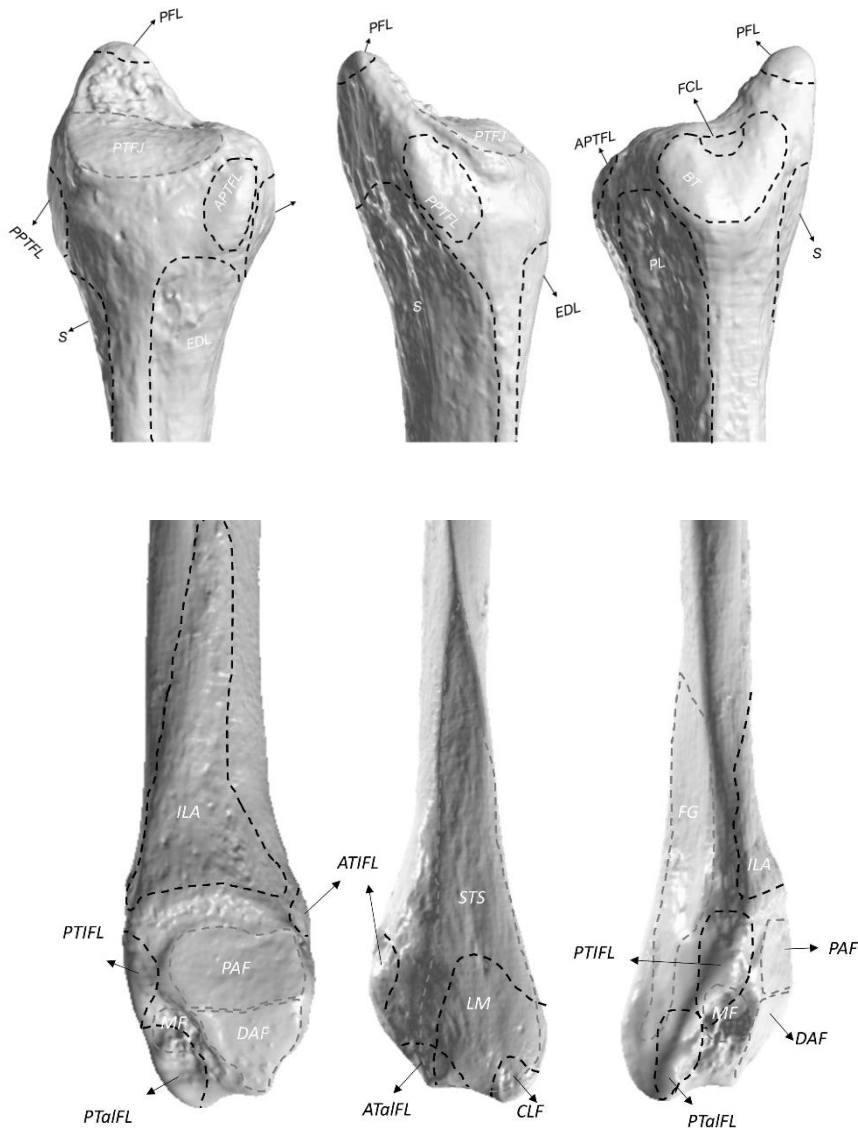


Figure 2. Anatomy of the proximal fibula (top). From left to right, medial, posterior and anterolateral view. Abbreviations: PTFJ: proximal tibiofibular joint; APTFL: anterior proximal tibiofibular ligaments; PPTFL: anterior proximal tibiofibular ligaments; PFL: popliteofibular ligament; BT= m. *biceps femoris* tendon attachment; FCL= insertion of the fibular collateral ligament; PL= m. *peroneus longus* origin; S= m. *soleus* attachment; EDL= m. *extensor digitorum longus* insertion. Anatomy of the distal fibula (bottom). From left to right, medial, lateral and posteromedial view. Abbreviations: PAF: proximal fibulotalar articular surface; DAF distal fibulotalar articular surface; MF: malleolar fossa; STS: subcutaneous triangular surface; LM: lateral malleolus; ILA: interosseous ligament attachment area; FG: fibular groove; ATIFL: anterior tibiofibular ligament; PTIFL: posterior tibiofibular ligament; ATaIFL: anterior talofibular ligament; PTaIFL: posterior talofibular ligament; CLF: calcaneofibular ligament.

1.2 Fibular biomechanics and kinematics

The fibula has a role in load sharing, laterally supporting the leg and the ankle (Auerbach *et al.*, 2011). Experimental studies on humans showed that the fibula bears between 8-19% of the total load on the leg in adults depending on foot positions: higher when the foot is flexed and/or inverted and everted (Funk *et al.*, 2004; Goh *et al.*, 1992; Lambert, 1971; Takebe *et al.*, 1984). When the foot is dorsiflexed, the load on the fibula increases (Barnett & Napier, 1953; Carleton, 1941). The fibula bears significant compressive loads when the foot is everted. When the ankle is inverted it experiences minimal compressive forces and occasional tensile forces (Funk *et al.*, 2007). The proportion of fibular load-sharing in the leg increases while engaging in high-impact activity and traversing uneven terrain (Rantalainen *et al.*, 2010; Sparacello *et al.*, 2014), thanks to the interosseous membrane which transmits a higher amount of forces from the tibia (Minns & Hunter, 1976; Skraba & Greenwald, 1984; Thomas *et al.*, 1995). The fibula also mitigates the torsional forces from the femur to the tibia (Thambyah & Pereira, 2006). The fibula, when subjected to bending and torsion, experiences comparable stiffness and strain distribution to those of the femur and tibia (Cristofolini *et al.*, 2010). During the gait cycle, at the initial phase of heel strike, kinematic studies showed that the fibula shifts inferiorly, with simultaneous lateral and posterior translation. This motion creates a deeper ankle mortise for the talus providing support to the talocrural joint in dorsiflexion and maximal stability when weight-bearing

forces are the greatest, while also translating them into tension forces through the interosseous membrane (Weinert *et al.*, 1973; Lundgren, 1989; Svenson, 1989; Bozkurt *et al.*, 2005). From heel strike to stance, after dorsiflexion, the fibula (and the foot) everts and externally rotates relatively to the tibia (Wang *et al.*, 2015). During the following stance phase, fibular internal/external rotation and vertical translation occur (Wang *et al.*, 2015). Indeed, Weinert & colleagues (1973) previously documented the distal migration of the fibula during stance, and Scranton & colleagues (1976) showed that this motion could occur as the flexor muscles of the foot contract during the stance phase. From the stance phase to toe-off, the fibula (and the foot) plantarflexes, inverts and rotates internally to the tibia (Wang *et al.*, 2015). During the following toe-off phase, the fibula translates medially and externally rotates, relatively to the tibia, as the degree of plantarflexion increases (Bozkurt *et al.*, 2008).

Fibular motion occurring during foot dorsiflexion from a plantarflexed position (Weinert *et al.*, 1973; Lundgren, 1989; Svenson, 1989; Borzkurt *et al.*, 2005, Kärrholm *et al.*, 1985; Ahl *et al.*, 1987) is accentuated compared to those induced by foot supination/pronation, which is minimal (Lundgren, 1989; Svenson, 1989; Ahl *et al.*, 1987).

Despite the role of the fibula in load bearing and support during gait and different foot postures, the resection of the fibula diaphysis have no significant effect on kinematic or kinetic variables of gait (Maurer-Ertl *et al.*, 2012), except for the longer double support phase (Chou *et al.*, 2009). However, the resection of the distal segment of the fibula may result in ankle lateral instability (Jung *et al.*, 2012).

1.2.1. Proximal tibiofibular joint biomechanics and kinematics

The PTFJ is primarily involved in the dissipation of torsional stresses applied at the ankle joint (Ogden, 1974a; 1974b) while also contributing to the dissipation of lateral tibial bending moments and tensile forces, rather than compressive weight bearing (Ogden, 1974a; 1974b). Indeed, Evans & Bang (1966) found that proximal fibulae were stronger (ultimate tensile and single shearing strength), stiffer (modulus of elasticity), and had a greater tensile strain (% elongation) in comparison to femurs, (see also Preuschoft, 1970). The PTFJ

rotates externally when the ankle is in dorsiflexed position (Andersen, 1985; Bozkurt *et al.*, 2003). Lambert (1971) suggested approximately one-sixth of the static load applied at the ankle is transmitted to the PTFJ. The PTFJ is also involved in the anteroposterior shifting of approximately 1 cm in both directions according to flexion/extension movements at the knee, moving anteriorly when the knee is in a flexed position and posteriorly when the knee is extended (Ogden, 1974a; 1974b; Alves-da-Silva *et al.*, 2019). Finally, anteroposterior movement of the fibular head during internal and external rotation of the lower limb occurs (Scott *et al.*, 2007) (**Figure 3; Table 1**).

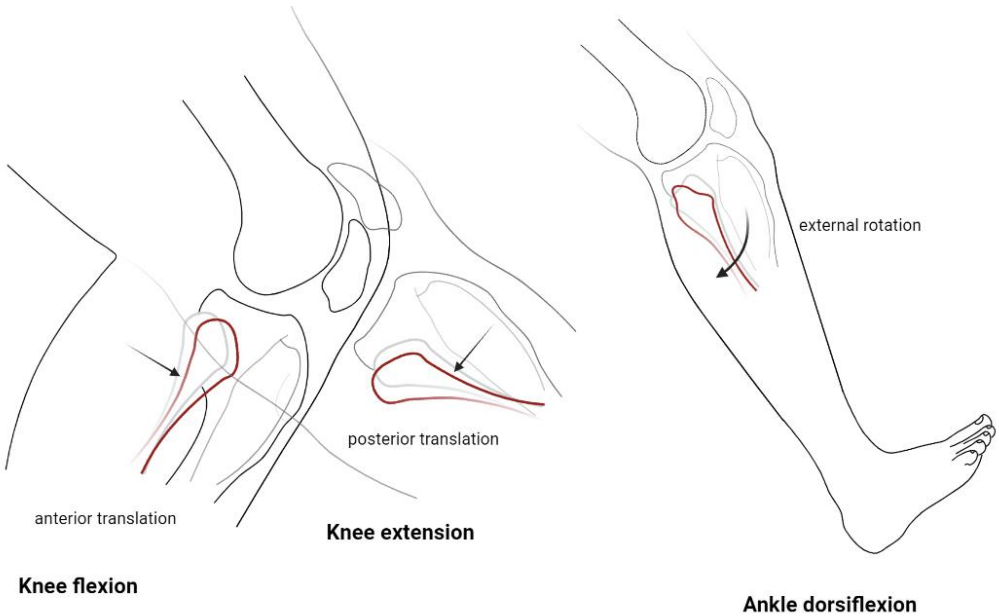


Figure 3. Schematic representation of proximal fibular motions during knee flexion and extension and ankle dorsiflexion.

1.2.2 Talocrural and tibiofibular joint biomechanics and kinematics

Load on the distal fibula (produced by talofibular contact within the talocrural joint) varies with ankle position. Maximum loads occur at full dorsiflexion and eversion (Funk *et al.*, 2004; Goh *et al.*, 1992). Barnett & Napier (1952) highlighted fibular axial motion, as well as a change in the axis of rotation of the talus with ankle flexion. When the foot dorsiflexes, greater joint surfaces congruity among tibia, fibula and talus is achieved compared to plantarflexion (Calhoun *et al.*, 1994). Indeed, in the loaded ankle, articular contact restraint is greater than ligament restraint for dorsiflexion stability of the ankle (Smith & Reischl, 1988; Stormont *et al.*, 1985). Additionally, eversion of the ankle generates an increase in contact at the talofibular facets (Calhoun *et al.*, 1994). Lambert (1971) pointed out that since the talofibular articular area is approximately oblique, compressive forces transmitted across the joint have a horizontal and axial component, consisting of tension through the distal tibiofibular ligaments and compression on the fibula respectively.

Regarding the tibiofibular syndesmosis, relative to the tibia, the distal fibula glides medially and anteriorly, and rotates internally in plantarflexion. In dorsiflexion, the distal fibula moves laterally and posteriorly, and rotates externally (Hu *et al.*, 2019). From plantarflexion to dorsiflexion of the ankle, the average mortise widening is about 1 mm (Lofvenberg *et al.*, 1990). Additionally, under axial loading, the fibular lateral malleolus migrates distally and laterally to the tibia at the tibiofibular joint (Wang *et al.*, 1996; Weinert *et al.*, 1973; Lundgren, 1989; Svenson, 1989; Borzkurt *et al.*, 2005). Foot inversion and eversion have minimal effect on fibular motion (Lundgren, 1989; Svenson, 1989; Ahl *et al.*, 1987) (**Figure 4; Table 1**).

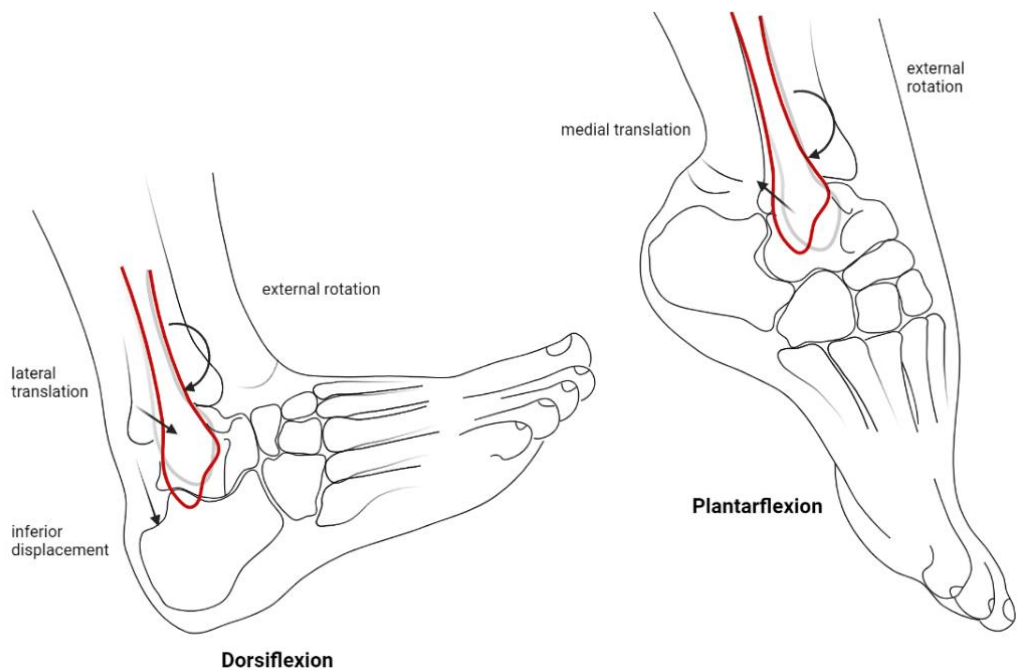


Figure 4. Schematic representation of distal fibular motions during ankle dorsiflexion and plantarflexion.

	ankle dorsiflexion/ plantarflexion	ankle inversion/eversion	knee flexion/extension	leg rotation
proximal fibula	external/internal rotation; little displacement	moderate displacement	anteroposterior translation	anteroposterior translation
distal fibula	anteroposterior, mediolateral and superoinferior translation; external/internal rotation	little mediolateral displacement	little to no displacement	external/internal rotation; mediolateral and anteroposterior translation

Table 1. Summary of fibular motion during ankle and knee flexion and leg rotation.

1.3. Background: intraspecific, interpopulation and interspecific variation

1.3.1. Studies on intraspecific variation on human leg properties in relation to sexual dimorphism

The shape and size of leg bones are consistently analysed for sex estimation in anthropology, with both bioarchaeological and forensic applications (e.g., İşcan & Miller-Shaivitz, 1984; Kranioti & Apostol, 2015; Brzobohatá *et al.*, 2016; Fasemore *et al.*, 2018). It is assumed that sexual dimorphism of human lower limbs is influenced by several environmental and hormonal factors, as well as affected by the differential action of physical load, contributing to sexual variations of body shape and size/proportions (Ruff, 1987a). Generally, males display larger and heavier tibiae and fibulae, with more robust lower extremities than females (Brothwell, 1981; Ubelaker, 1989).

The human tibial diaphyseal shape and robusticity have been extensively studied with biomechanical and both traditional and virtual morphometrics approaches, and its characteristics are also adopted for sex assessment (Kimura, 1971; Ruff & Hayes, 1983; Carlson *et al.*, 2007; Brzobohatá *et al.*, 2014; 2015; 2016).

The fibula, however, remains scarcely analysed in studies of sexual dimorphism, although fibular metric variations have revealed their potential in distinguishing between males and females: studies including fibular metrics for sexual estimation report accuracies of 80–86% for multivariate analyses of fibular measurements in populations with different ancestries (Sacragi, Ikeda & Terada, 1993; Spradley & Jantz, 2011; Spradley, Anderson & Tise, 2015; Moore *et al.*, 2016; Krüger *et al.*, 2017). Singh & Singh (1976) tested the potential of the width of the distal epiphysis for sex discrimination, and concluded that this measure, together with fibular length and mid-shaft circumference correctly estimated sex with 99.7% accuracy. A possible limitation of such approaches may consist in their focus on gross anatomical fibular features, as they mostly concentrated on the fibular diaphysis (e.g., maximum length and maximum diameters), which are heavily influenced by body size.

Focusing of fibular distal epiphysis, Sacragi & Ikeda (1995) measured the talar articular surface, the malleolar fossa, and the lateral malleolus, revealing that

such measures correctly classified males at 90.1% and females at 91.4% accuracies. Similarly, Naidoo & colleagues (2015) examined different linear and angular dimensions of the talar facet, and found significant variations among males and females, with limited effect of ancestry.

Some studies examined measurements, taken considering the fibular nutrient foramen as reference, in South African populations, obtaining >70% accuracy (Naidoo *et al.*, 2015; Mazenganya & Billings, 2016; Fasemore *et al.*, 2018).

The fibular proximal epiphysis is rarely studied in anthropology for sex determination purposes, and only studies accounting for three-dimensional fibular shape variations included the proximal extremity, along other tibial and fibular regions, in their analysis (Zhang *et al.*, 2016; Tümer *et al.*, 2019; Nolte *et al.*, 2020; Audendart *et al.*, 2019; Bruce *et al.*, 2022).

Indeed, virtual morphometric approaches have the advantage of accounting for the whole bone shape and size variations, overcoming the possible limitation that may rise when focusing on single measurements only. Prior studies involving three-dimensional statistical shape models (SSM) of fibulae (within the tibio-fibular complex), considering both fibular extremities and the diaphysis, found that sex contributes only subtly to the differentiation of fibular morphologies, with larger proximal epiphyseal region in males compared to females, despite not significantly and with limited variance explanatory power (Bruce *et al.*, 2022). Tümer & colleagues (2019) did not find a consistent pattern of shape variation in relation to sex for the fibula. The 3DGM studies of the whole fibula (including diaphysis and both epiphyses) highlighted little to no morphological changes between sexes (Maas & Friedling, 2020). Males and females differed in fibular length (females shorter than males), but not in terms of fibular shape, which failed to distinguish the sexes (Maas & Friedling, 2020). To our knowledge, no prior work focused on fibular epiphyseal shape using a virtual morphometric approach.

1.3.2. Studies on interpopulation variation on human leg properties in relation to loading regimen

Studies on the tibia have explored the role of different loading patterns in connection to biomechanical properties and diaphyseal shape. High levels of terrestrial mobility were linked to particularly robust and anteroposteriorly

elliptical (according to cross-sectional geometric analyses) diaphyses at midshaft. While tibial robusticity provides information on the level of mobility of a human sample (Ruff, 1987b; Stock, 2006; Ruff & Hayes, 1983), the tibial diaphyseal shape (i.e., ellipticity) is an indicator of loading directionality: Shaw & Stock (2009) revealed that cross-country runners and field hockey players had significantly more robust tibiae than controls, with unique tibial shapes for each group linked to the different loading directions. The authors further highlighted the correspondence between tibial robusticity and specialized mobility patterns in a bioarcheological sample, fossil hominins, and modern athletes (Shaw & Stock, 2013). Moreover, several bioarcheological studies, approached tibial shape as an indicator of changes in mobility levels (Stock & Pfeiffer, 2001; Holt, 2003; Sladák, Berner & Sailer, 2006; Marchi, 2008; Marchi, Sparacello & Shaw, 2011;).

The structural properties and shape of the fibular diaphysis relative to the tibia in response to different loading patterns were also explored. Rantaleinen & colleagues (2010) documented a higher robusticity of the tibia and fibula with high-impact activities but also suggested that the tibia and fibula experience different loading responses despite their anatomical vicinity, depending on the amount of fibular weight-bearing. The authors hypothesize that this different response to loading is influenced by the complex interaction of tibiofibular kinematics at both proximal and distal tibiofibular joints (Rantaleinen *et al.*, 2010). Similarly, Marchi & Shaw (2011) documented a progressive increase in fibular robusticity from sedentary controls to runners to field hockey players, linked to a higher degree of ankle dorsiflexion and frequency of cutting movements in the latter groups. More recently, Auerbach & coworkers (2017) demonstrated that the cross-sectional properties of the tibia and the fibula vary with respect to their relative position to each other, hypothesizing that the two bones functions both complementarily and in single units.

Several studies confronted fibular structural properties among human archaeological samples to explore their role and correspondence to mobility patterns in bioarcheological contexts, comparing habitual activities of different past populations and from different geographical regions (Marchi, Sparacello & Shaw, 2011; Sparacello, Marchi & Shaw, 2014; Sparacello *et al.*, 2018;

Hagihara & Nara, 2016). Marchi, Sparacello & Shaw (2011) examined the impact of mountainous, uneven terrain on the fibular robusticity of two Italian Neolithic and Iron Age samples compared to medieval and modern populations inhabiting flat geographical areas. The authors found that fibular robusticity, either considered alone or paired with the tibia, is correlated to mobility level and is influenced (i.e., increases) by uneven terrain. Sparacello, Marchi & Shaw (2014) further documented higher relative fibular robusticity in a mountain-dwelling archaeological sample compared to samples from regions with flat terrain. Sparacello & coworkers (2018) examined the Mid to Late Upper Paleolithic – Mesolithic transition as documented by structural and shape variations in the tibiofibular complex correlating with mobility levels and types. They showed that individuals chronologically spanning this transition are comparable to modern athletes, documenting their high activity levels.

Little investigation has been performed on tibiae and fibulae epiphyseal shape. Regarding the study of tibial epiphyseal shape in skeletal samples, several investigations adopted a variety of methodological solutions to explore external shape morphologies for a variety of scopes (e.g., Latimer Ohman & Lovejoy, 1987; Stevens & Vidarsdóttir, 2008; DeSilva, 2009; Frelat *et al.*, 2012; 2017). Brzobohatá & colleagues (2014) adopted a 3DGM approach to explore variations of tibial extremities of archaeological populations belonging to different socioeconomic living conditions (high vs. low economic status). However, they did not identify a statistically significant correspondence between tibial epiphyseal shape and socioeconomic category, though a possible trend in distal epiphyseal shape was detected.

To our knowledge, no previous work on fibular epiphyseal shape has tested the possible link between fibular epiphyseal morphology and loading regimen in an interpopulation analysis accounting for subsistence patterns, mobility, and lifestyle.

1.3.3. Studies on interspecific variation on human leg properties in relation to locomotor behavior in hominids

Numerous biomechanical studies have been performed on the diaphysis of the tibia of primates (Ruff, 1987b, 1989, 2002; Demes *et al.*, 2001; Marchi, 2007; Marchi, 2015a) and highlighted the role of tibial properties in discriminating

among locomotor behavior among taxa.

Studies conducted on the relative (to the tibia) fibular robusticity [as evidenced by cross-sectional geometry (CSG) analysis] of the human and nonhuman primate fibula found a relationship between fibula/tibia diaphyseal strength ratios and degree of arboreal locomotion in several catarrhines (Marchi, 2007; 2015a; McLean & Marzke, 1994). The authors found that the more arboreal taxa were characterized by relatively more robust fibulae at midshaft than were the more terrestrial ones (Marchi, 2007; 2015a). Orangutans and chimpanzees had relatively more robust fibulae than gorillas or human. It was proposed that this relationship may reflect the different degree of mobility of the fibula at the ankle joint due to the different range of foot dorsiflexion/plantarflexion and inversion/eversion generated during locomotion using arboreal vs. terrestrial substrates in living and fossil species (Barnett & Napier, 1953; Carleton, 1941; DeSilva, 2009; Latimer *et al.*, 1987; Stern & Susman, 1983). Recent studies also showed the importance of studying tibial and fibular diaphyseal strength to further our understanding of the degree of arborealism of early hominins (Marchi *et al.*, 2019).

The morphology of tibial and fibular epiphysis was also investigated. Tibial epiphyseal shape was explored to assess the link between external shape tibial morphologies at the extremities and locomotor strategy and evolutionary timing in living and extant hominids (e.g., Latimer, Ohman & Lovejoy, 1987; DeSilva, 2009; Frelat *et al.*, 2017; DeSilva & Throckmorton, 2010; Venkataraman *et al.*, 2013; Sylvester, 2013). A squared talocrural joint shape is found in humans, uniformly distributing forces across the joint in bipedal locomotion with a perpendicular orientation to the long axis of the tibial shaft. In great apes, the joint is trapezoidal with an anterior tilt to accommodate the foot in extreme dorsiflexion during climbing (DeSilva, 2009; DeSilva & Throckmorton, 2010; Venkataraman *et al.*, 2013; Latimer, Ohman & Lovejoy, 1987; Sorrentino *et al.*, 2020a; Turley, White & Frost, 2015). Some studies investigated the morphology of fibular distal end. Previous studies performed using traditional morphometrics showed that great apes possess a more anteriorly oriented lateral malleolus and more downward facing fibulotalar articular facets as well as a more craniocaudally elongated STS than humans

(Marchi, 2015b; Stern & Susman, 1983). A recent 3DGM study revealed that the distal fibular morphology can be used to segregate arboreal vs. terrestrial locomotor patterns within extant hominids (Marchi *et al.*, 2022). To our best knowledge, no comparative study on proximal fibular shape and metrics among living hominid taxa has never been performed.

1.4. Aims and research hypotheses

This work explores, describes, and quantify the morphological variations of fibular extremities in *H. sapiens*, spanning Mid-Late Upper Paleolithic to present, and living non-human hominids, utilizing a 3DGM approach.

The aims of this doctoral thesis are threefold:

- **Aim I.** Our first objective is to investigate intraspecific variations of fibular epiphyseal shape, form, and size in *H. sapiens*, documenting sexually dimorphic features in the fibular proximal and distal extremities in three populations from Italy and South Africa, belonging to identified modern human skeletal collections (19th-20th century). Our goal is to develop a new 3DGM method to be implemented for human sex assessment. In detail, based on previous literature (e.g., Maas & Friedling, 2020; Sacragi and Ikeda, 1995; Bruce *et al.*, 2021), we expect (a) that fibular epiphyseal form (size + shape) will be sexually dimorphic, likely reflecting functional differences (body shape, size, and proportions), while shape alone will be less informative. We also expect (b) differing patterns of epiphyseal size dimorphism due to differentiation of body size among Italian and South African groups, but differences in the sexual dimorphism between the populations in shape alone.
- **Aim II.** Our second objective is to explore interpopulation shape variability of fibular proximal and distal extremities among Italian populations dated from the Mid-Late Upper Palaeolithic to 20th century, including populations with different subsistence strategies with progressively declining activity levels, and thus comprising hunter-gatherers, agro-pastoralists and pre- and post-industrialists. Based on previous literature (e.g., DeSilva, 2009; DeSilva & Throckmorton, 2010; Holowka *et al.*, 2017 Sorrentino *et al.*, 2020b;

Marchi, Sparacello & Shaw, 2011; Marchi *et al.*, 2011; Sparacello, Marchi & Shaw, 2014; Sparacello *et al.*, 2018), we expect: (a) that fibular morphology would account for the different functional requirements (e.g., frequent flexion, strong eversion, mobile joints) of the lower limb among the most and least active groups in relation to subsistence (foragers vs. sedentary city dwellers); (b) that population with intermediate mobility (agriculturalist) would show a mosaic morphology, variably falling in-between extremes depending on their habitual frequency of other activities (e.g., military activity). Moreover, we will test possible shape variations related to terrain type and shoe use to test fibular shape as a potential indicator of finer cultural aspects, expecting (c) to find morphologies that are consistent with the requirements of frequent ankle flexion attained during traversing uneven terrain in mountain dwellers and a flexible ankle configuration in barefoot populations, compared to the vertically loaded, static ankle configurations of city dwellers.

- **Aim III.** Our third objective is to provide a detailed comparative assessment of shape variation of the proximal and distal fibula in extant humans and great apes using 3DGM to detail the variation of proximal fibular shape in each taxon and investigate a possible link with locomotor and positional behaviors. Based on previous studies outlined above, we expect that the shape of the fibula in extant hominids will significantly differ between humans and great apes because the different locomotor and postural repertoires entail different loading regimens applied at the knee in flexion (Aiello & Dean, 1990; Isler, 2005; Scott *et al.*, 2007) and at the ankle in dorsiflexion and eversion (Ogden, 1974a, 1974b; Susman and Stern, 1983; Latimer, Ohman & Lovejoy, 1987; Marchi *et al.*, 2015b; Marchi *et al.*, 2022).

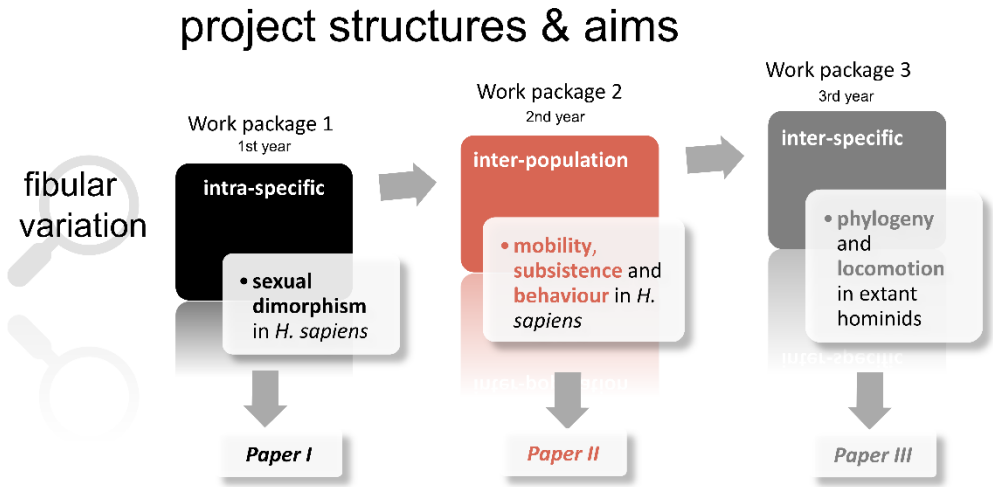


Figure 5. Graphic representation of the structure of the work packages, aims and results.

A distinct work package (**Figure 5**) was dedicated to each of these aims and reflect the organization of the results contained in thesis (**Chapter 3**). Each year of the three-year-project was devoted to the implementation of these three aims.



CHAPTER 2

Materials and Methods

CONTENTS

- THE SAMPLE
 - TEMPLATE
 - 3D GM
 - METHODOLOGY
- PAPER: MESH
COMPARABILITY
-

2. Materials and Methods

2.1. The sample

2.1.1. The identified sample

The human identified sample differently utilized in the Methodological Paper, as well as Paper I, II and II, consists of 136 left fibulae (Table 2) belonging to late 19th – early 20th century populations including South Africans (N = 17) from the Raymond A. Dart Collection of Human Skeletons at the University of the Witwatersrand, Johannesburg, South Africa (SA) (Dayal *et al.*, 2009), and Italians (two populations from the regions of Emilia Romagna (ER), (N = 47); and Sardinia (SAR), (N = 72) belonging to individuals from the Modern Human Identified Skeletal Collection of the University of Bologna (Italy) who died between 1898 and 1944 (Belcastro *et al.*, 2017). For both collections, sex is known for all included individuals from hospital or cemetery records, as well as other archival sources (e.g., birth certificates). Age-at-death spans between 17-90 years.

Population	Females	Males	Total
ER (Bologna, Parma)	24	23	47
SAR (Sassari, Cagliari, Nuoro)	29	43	72
SA (Johannesburg and surroundings)	10	7	17
Total	63	73	136

Table 2. Identified sample composition by sex.

2.1.2. The archeological human sample

The archaeological sample, analyzed for interpopulation variation of loading regimen (Paper II), consists of human skeletal fibulae belonging to 69 individuals from 17 Italian archaeological sites grouped chronologically spanning Mid-Late Upper Paleolithic to the 20th century (**Table 3**). Individuals of both sexes are included, and age-at-death spans between young to old adults (Buikstra & Ubelaker, 1989).

Population	Chronology	Subsistence	Terrain	Shoe use	F	M	ND	Tot
ROC (Roccapelago)	Modern Age (17 th -18 th century CE)	agriculturalists/herders	mountainous	hard-soled	-	-	10	10
OR (via Orfeo)	Early Modern Age (14 th -16 th century CE)	pre-industrial, sedentary	flat	soft-soled	4	4	-	8
VCN (Vicenne)	Early Middle Ages (7 th -8 th century CE)	agriculturalists/military activity	hilly	soft-soled	2	2	-	4
SUA (Suasa)	Roman Late Imperial Age (1 st -3 rd century CE)	agriculturalists	hilly	soft-soled	5	3	-	8
CER (Casalecchio Ceretolo)	Second Iron Age (4 th -3 th century BC)	agriculturalists/military activity	hilly	soft-soled	-	2	-	2
ODN (Olmo di Nogara)	Middle Bronze Age (1500-1300 BC)	agriculturalists/military activity	flat	soft-soled	2	5	-	7
NEO (Arene Candide, Grotta Pollera, Arma dell'Aquila, Bergeggi)	Neolithic (6000-4500 BCE)	agriculturalist/herders	mountainous	soft-soled	3	10	2	15
UP (Arene Candide, Barma Grande, Grotta dell'Uzzo, San Teodoro, Grotta d'Oriente, Mezzocorona, Mondeval de Sora)	Mid Upper Palaeolithic-Mesolithic (24.000-7500 BP)	hunter-gatherers/fish foragers	mountainous	barefoot/soft-soled	4	11	-	15
Total					20	37	12	69

Table 3. Archeological sample composition by population, chronology, sex and categories of subsistence, terrain type and shoe use.

ROC samples are hosted at the Department of Cultural Heritage of University of Bologna and curated by Museo Delle Mummie of Roccapelago and Soprintendenza Archeologia, Belle Arti e Paesaggio per la città metropolitana di Bologna e le province di Modena, Ferrara e Reggio Emilia. OR, SUA, VCN and CER samples are part of the osteological collections belonging to the Laboratory of Physical Anthropology at the Department of Biological, Geological and Environmental Sciences at University of Bologna. ODN sample are hosted at the Department of Biology at University of Pisa. NEO samples are part of the collections hosted by the Museo di Archeologia Ligure, Genova, the Museo Archeologico del Finale, Finale Ligure, and the Museo di Storia Naturale – Sezione di Antropologia e Etnologia, Università degli Studi di Firenze and managed by Soprintendenza Archeologia, Belle Arti e Paesaggio per la città metropolitana di Genova e le province di Imperia, La Spezia e Savona. The UP sample are hosted by several institutions: the Ligurian samples are part of the collections of the Museo di Archeologia Ligure, Genova, the Museo Archeologico del Finale, Finale Ligure, and the Museo di Storia Naturale – Sezione di Antropologia e Etnologia, Università degli Studi di Firenze, the Museo Preistorico Nazionale dei Balzi Rossi, Ventimiglia, and are managed by Soprintendenza Archeologia, Belle Arti e Paesaggio per la città metropolitana di Genova e le province di Imperia, La Spezia e Savona. The samples from Trentino and Veneto are hosted by the MuSe Museo della Scienza di Trento and Museo di Storia Naturale di Verona, and managed by Soprintendenza Archeologia del Veneto, and the Soprintendenza per i Beni Culturali della Provincia Autonoma di Trento. The Sicilian samples are hosted by the Museo Salinas, and the Museo di Geologia “G.G. Gemmellaro”, managed by Polo regionale di Palermo per i Parchi e i Musei Archeologici.

2.1.3. The non-human sample

The non-human sample, analyzed for interspecific analysis of locomotor behavior among extant hominid taxa (Paper III and report on distal fibulae), includes 57 fibulae belonging to 15 *Gorilla* (*Gorilla gorilla* = 12; *Gorilla beringei* = 3), 17 *Pongo* (*Pongo abelii* = 8; *Pongo pygmaeus* = 9), and 25 *Pan* (*Pan troglodytes* = 24; *Pan paniscus* = 1) (**Table 4**). All fibulae belong to adult individuals of both sexes.

The digital models of these fibulae were obtained by several institutions: the American Museum of Natural History (via www.morphosource.org); the National Museum of Natural History (via www.morphosource.org); the Collection of Anthropology of the Museal System of University of Bologna; the State Zoological Collection in Munich; the University of Zurich; the Primate Research Institute’s Digital Morphology database of Kyoto University (via KUPRI database <http://dmm.ehub.kyoto-u.ac.jp/dmm/WebGallery/index.html>).

Taxon	n	Institution	Sex		
			Males	Females	Unknown
<i>Pan troglodytes</i>	24	AMNH, NMNH, UZH, SZCM, KUPRI, UNIBO	15	7	2
<i>Pan paniscus</i>	1	AMNH	-	1	-
<i>Gorilla gorilla</i>	12	AMNH, UZH, SZCM, KUPRI	8	4	-
<i>Gorilla beringei</i>	3	AMNH, NMNH	2	1	-
<i>Pongo pygmaeus</i>	9	AMNH, NMNH, UZH, SZCM,	3	6	-
<i>Pongo abelii</i>	8	NMNH, UZH, SZCM, KUPRI	7	1	-
Total	57		35	20	2

Table 4. The composition of the non-human sample by taxa and sex. Abbreviations: AMNH: American Museum of Natural History (via www.morphosource.org); NMNH: National Museum of Natural History (via www.morphosource.org); UNIBO: Human Identified Skeletal Collection of Bologna; SZCM: State Zoological Collection in Munich; UZH: University of Zurich; KUPRI: Primate Research Institute’s Digital Morphology database.

2.2. Sample acquisition

2.2.1 The digitalization of the identified samples

The acquisition of the SA fibulae was performed at the Microfocus X-Ray Computed Tomography facility of the University of Witwatersrand (Johannesburg, South Africa) on a Nikon Metrology XTH 225/320 LC (Voltage 70 kV, current 120 μ A, no filter used, pixel size 120 μ m). The acquisition of the fibulae of ER and SAR was carried out at Istituto Ortopedico Rizzoli (Bologna,

Italy) utilizing a Revolution Discovery CT dual energy, with GSI Revolution and HD Revolution configurations (Voltage 100 kV, current 360 μ A, standard filter, slice thickness and acquisition interval at 0.625 mm, followed by a reconstruction with monochromatic beams at 40 keV, using the “Detail” filter, voxel size ranging from 0.39 to 0.507 x 0.625 mm). The reconstructed DICOM (16-bit gray-scale, signed) images were then processed with Avizo 9.2 (Thermo Fisher Scientific) for image segmentation with half-maximum height (HMH) protocol outlined by Spoor & coworkers (1993) following Coleman & Colbert (2007). An isosurface was then generated.

A subsample of 20 Italian individuals of ER were digitized with ARTEC Space Spider 3D (Luxembourg), a mobile and structured light laser scanner (point-accuracy: 0.05 mm; mesh resolution: 0.1 mm), housed at the BONES Lab in the Department of Cultural Heritage of the University of Bologna (Artec Group, 2013). The acquisition took place in two recording sessions for each specimen, then integrated with the scanner software (Artec Studio 9) after first cleaning and manual alignment. These aligned scans were globally registered. A single polygonal 3D mesh was created with the Sharp Fusion algorithm, finally generating the 3D surface model exported in .stl (Little Endian) format (Artec Group, 2013).

SAR, ER and SA samples consist of dry bone samples.

2.2.2. The digitalization of the archaeological samples

ROC, OR, VCN, SUA, CER, and ODN samples were digitized with ARTEC Space Spider 3D (Luxembourg), housed at the BONES Lab in the Department of Cultural Heritage of the University of Bologna, a mobile and structured light laser scanner (point-accuracy: 0.05 mm; mesh resolution: 0.1 mm) following the protocol outlined above for the subsample of ER identified individuals (Artec Group, 2013).

NEO and UP samples were digitized using the structured light scanner DAVID® SLS-2 (DAVID Group 2007-2015).

ROC, OR, VCN, SUA, CER, and ODN samples are dry bones.

2.2.3. The digitalization of the non-human samples

Three-dimensional surface meshes of the fibula belonging to one chimpanzee housed at the University of Bologna (UNIBO) institution were digitized at the

Istituto Ortopedico Rizzoli (Bologna, Italy) utilizing a Revolution Discovery CT dual energy following the protocol outlined for ER and SAR identified sample. Three-dimensional surface meshes of ape fibulae belonging to the State Zoological Collection in Munich (SZCM), Germany, and the University of Zurich (UZH), Switzerland, were obtained using medical CT scanning. The SCM specimens were collected at the Munich Institute for Radiology Ludwig Maximilian University (Munich, Germany) on a GE Discovery CT750 HD medical CT scanner (slice thickness 0.625 mm, slice increment 0.3 mm, voltage 120 kV, X-ray tube current 99 mA, reconstructing algorithm bone, pixel size 460 μm). The UZH specimens were collected at the University Hospital of Zurich (Zurich, Switzerland) on a Siemens Somatom Definition Flash (slice thickness 0.6 mm, slice increment 0.3 mm, voltage 120 kV, current 19 mA, reconstructing algorithm bone, pixel size 600 μm). Similar to the UNIBO sample, the SZCM and UZH samples were segmented utilizing the HMM segmentation protocol detailed previously. All UNIBO, UZH, and SZCM samples consisted of dry bone specimens.

Selected fibulae belonging to the Division of Mammals, National Museum of Natural History, Smithsonian Institution, and the Mammal Collections at the American Museum of Natural History were obtained via MorphoSource (<https://www.morphosource.org/>). The specimens include dry bone samples, and the digital models were obtained either by laser scanning or CT- scanning. Other ape specimens were obtained via Digital Morphology (KUPRI) from the Primate Research Institute's (PRI) collection of CT scans. For these specimens, scan resolution (X, Y) ranged from 0.133-0.761 mm, with a slice thickness (Z) of 1-2 mm.

The specimens included dry bones, fresh cadavers, and other samples with frozen or immersed soft tissue.

2.3. Geometrics morphometrics

The analysis of morphological bone variability benefits from new virtual methods developed a set of techniques known as geometric morphometrics (Rohlf & Slice, 1990). Such approach, compared to traditional morphometric analysis using linear dimensions, allows the analysis of full, complex geometries of a shape, which are retained and described by landmark data

(O'Higgins, 2000). Landmarks are fixed, anatomical points that are homologous in all specimens of a data set. Sets of landmarks can be recorded in two (x- and y coordinates) or three dimensions (x-, y-, z-coordinates) (O'Higgins, 2000; Adams, Rohlf & Slice, 2004; Slice, 2006; Baab *et al.*, 2012). Complex structures areas are also captured by semilandmarks, describing curves and surfaces. These points become geometrically homologous among all configurations in a given dataset, thanks to the sliding procedures based on thin plate spline (TPS) interpolation, and they can be analyzed along with traditional landmarks (Gunz & Mitteroecker, 2013).

2.3.1. Template design

A template of 142 landmarks and semilandmarks, created in Viewbox v. 4.0, captured both fibular extremities, covering major muscle, ligament, and tendon attachment sites and articular surfaces (**Figure 6, Table 5**). The template configuration was applied to all target samples in Viewbox v. 4.0. Semilandmarks were allowed to slide on curves and surfaces, minimizing TPS bending energy between the targets and the template (Slice, 2006) so that semilandmarks could be considered geometrically homologous among specimens (Gunz, Mitteroecker & Bookstein, 2006; Gunz & Mitteroecker, 2013).

Landmarks	Definition
L1	Point where the <i>fibular anterolateral border</i> divides into two ridges: the proximal apex of the <i>subcutaneous triangular surface</i> (STS)
L2	Most medial point of the medial border of the STS
L3	Most lateral point of the lateral border of the STS
L4	Most distal point of the <i>lateral malleolus</i> in anterior view
L5	Most distal point of the posterior border of the <i>malleolar fossa</i>
L7	Most anterior point on the anterior border of the <i>proximal fibular-talar articular facet</i> (PAF)
L8	Point between the anterior border of PAF and the <i>anterior border of distal fibular-talar articular facet</i> (DAF)
L9	Most distal point of DAF
L10	Most proximal point on the posterior border of DAF
L12	Most posterior point of the proximal border of PAF
L13	Most proximal point of <i>proximal tibio-fibular articular facet</i>

L14	Most proximal point of <i>interosseous tibio-fibular ligament</i> (ILA) insertion	
L15	Most proximal point on styloid process of fibular head in medial view	
L16	Most antero-proximal point on anterior border in medial view (above fibular neck)	
L17	Most postero-proximal point on posteromedial border in medial view (above fibular neck)	
L18	Most postero-proximal point on posterior border in lateral view (above fibular neck)	
Curves	Definition	Number
C_1->2	Medial border of the <i>subcutaneous triangular surface</i> (STS)	5
C_1->3	Posterior border of the STS	7
C_7->8	Anterior border of the <i>proximal fibular-talar articular facet</i> (PAF)	1
C_8->9	Anterior border of the <i>distal fibular-talar articular facet</i> (DAF)	1
C_9->10	Posterior border of the DAF	1
C_10->12	Posterior border of the PAF	1
C_8->10	Border between the PAF and DAF	1
C_13->13	Outline of <i>proximal tibio-fibular articular facet</i>	6
C_7->12	Proximal border of the PAF	2
Surfaces	Definition	Number
SSML_malleolar fossa	Surface of the <i>malleolar fossa</i> , attachment site of the <i>transverse tibiofibular</i> and <i>posterior talofibular ligaments</i> .	7
SSML_ILA	Attachment surface of <i>interosseous tibio-fibular ligament</i> and part of <i>interosseous membrane</i> (ILA)	13
SSML_fibular groove	Groove for tendons of m. <i>peroneus longus</i> and m. <i>tertius</i> and attachment site of <i>posterior tibiofibular ligament</i> .	13
SSML_STS	<i>Subcutaneous triangular surface</i> (STS)	24
SSML_FiTal1Ar	<i>Proximal fibular-talar articular facet</i> (PAF)	4
SSML_FiTal2Ar	<i>Distal fibular-talar articular facet</i> (DAF)	3
SSML_head	<i>Proximal tibio-fibular articular surface</i>	5
SSML_prox_ep	Surface of <i>proximal epiphysis</i>	32

Table 5. Landmark and semilandmark template configuration for fibular proximal and distal epiphysis. For each landmark and semilandmark, name, definition and number are reported. See Figure 6 for visual display and landmark positioning on the fibular ends.

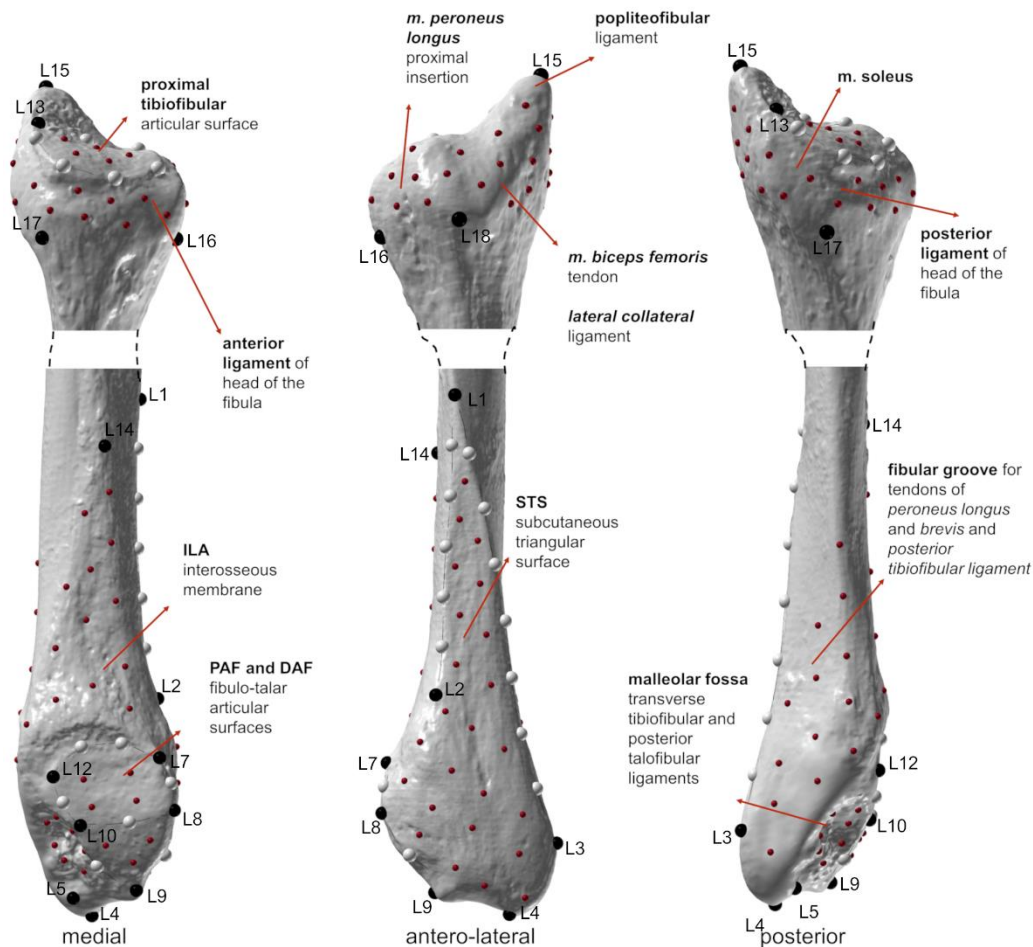


Figure 6. The template configuration on a human left proximal fibula in medial view (left), antero-lateral view (centre) and posterior view (right). Black dots are fixed landmarks, grey dots are curve semilandmarks and red dots are surface semilandmarks. See Table 5 for definition of numbered landmarks and curve.

2.3.2. 3DGM analysis

Raw Cartesian coordinates corresponding to each landmark and semilandmark, obtained after the application of the template to all samples and the sliding procedure between the template and the target, were imported in R v.4.0.3 (R Core Team, 2020). A further sliding of semilandmarks was performed against recursive updates of the Procrustes consensus while computing two Procrustes superimpositions via Generalized Procrustes Analysis (GPA), one GPA for each epiphysis after sub-setting the respective coordinates accordingly. Raw coordinates, separated for each epiphysis, were meanwhile converted into standardized, scaled, centered, and oriented shape coordinates (i.e., Procrustes coordinates) (Slice, 2006; Rohlf & Slice, 1990) using the *gpagen()* function in the R package "geomorph" version 3.3.2 (Adams *et al.*, 2018). Outliers from Procrustes consensus were found utilizing the function *plotOutliers()* provided in this package and removed.

Procrustes coordinates for each epiphysis were then subjected to a Principal Component Analysis (PCA) to explore shape variations among sexes, populations, categories and taxa (Schlager, 2017).

Visualization of shape changes along the principal axes and means of genera or categorical groups were obtained by TPS deformation (Bookstein, 1991) of the Procrustes grand mean shape surface utilizing the R package "Morpho" v. 2.8 (Schlager, 2017). In addition, visualizations of the mean shape distances among mean configurations of either population, category or taxa and Procrustes consensus were computed utilizing the function *localmeshdiff()* from the package "Arothron" v. 2.0.3 (Melchionna *et al.*, 2020; Profico *et al.*, 2021).

Procrustes ANOVA was utilized to evaluate shape differences among different populations and categories, sexes and taxa by calculating Procrustes distances among specimens using a residual randomization procedure (RRPP = T, iterations = 1,000), with the R package "geomorph" v. 3.3.2 (Adams *et al.*, 2018). Procrustes distance distribution was visualized by computing density values of Procrustes distances of each specimen to the Procrustes consensus. Additionally, principal component (PC) scores were evaluated and compared using a one-way ANOVA, with subsequent *posthoc* pairwise comparisons.

The centroid size (CS) was also calculated and used as a proxy of the size of both fibular extremities separately (Slice, 2006). Differences in CS among configurations were evaluated using ANOVA and subsequent *posthoc* tests and visualized in relative box plots. Finally, the form-space (i.e., shape plus size) PCA was computed by augmenting the Procrustes shape coordinates of each dataset of Procrustes coordinates by the natural logarithm of CS ($\ln CS$) (Klingenberg, 2016). Linear correlation among CS and the PC scores along the first two shape PCs was assessed by a Pearson's correlation test, to assess whether the distribution of individuals in the shape space PCA plot was influenced by size.

PAPER

Methodology

CONTENTS

Pietrobelli A, Sorrentino R, Notariale V, Durante S, Benazzi S, Marchi D, Belcastro MG (2022), Comparability of skeletal fibulae surfaces generated by different source scanning (dual-energy CT scan vs. high resolution laser scanning) and 3D geometric morphometric validation. *Journal of Anatomy* 241, 667 – 682

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METHOD

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Comparability of skeletal fibulae surfaces generated by different source scanning (dual-energy CT scan vs. high resolution laser scanning) and 3D geometric morphometric validation

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Title:

Comparability of skeletal fibulae surfaces generated by different source scanning (dual-energy CT scan vs. high resolution laser scanning) and 3D geometric morphometric validation

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Abstract

This work aims to test accuracy and comparability of 3D models of human skeletal fibulae generated by clinical CT and laser scanner virtual acquisitions. Mesh topology, segmentation and smoothing protocols were tested to assess variation among meshes generated with different scanning methods and procedures, and to evaluate meshes-interchangeability in 3D geometric morphometric analysis.

A sample of 13 left human fibulae were scanned separately with Revolution Discovery CT dual energy (0.625 mm resolution) and ARTEC Space Spider 3D structured light laser scanner (0.1mm resolution). Different segmentation methods, including half-maximum height (HMH) and MIA-clustering protocols,

were compared to their high-resolution standard generated with laser-scanner by calculating topological surface deviations. Different smoothing algorithms were also evaluated, such as Laplacian and Taubin smoothing. A total of 142 semilandmarks were used to capture the shape of both proximal and distal fibular epiphyses. After Generalized Procrustes superimposition, the Procrustes coordinates of the proximal and distal fibular epiphyses were used separately to assess variation due to scanning methods and the operator error. Smoothing algorithms at low iteration do not provide significant variation among reconstructions, but segmentation protocol may influence final mesh quality (0.09–0.24 mm). Mean deviation among CT-generated meshes that were segmented with MIA-clustering protocol, and laser scanner-generated ones, is optimal (0.42 mm, ranging 0.35–0.56 mm). Principal component analysis reveals that homologous samples scanned with the two methods cluster together for both the proximal and distal fibular epiphyses. Similarly, Procrustes ANOVA reveals no shape differences between scanning methods and replicates, and only 1.38–1.43 % of shape variation is due to scanning device.

Topological similarities support the comparability of CT- and laser scanner-generated meshes and validate its simultaneous use in shape analysis with potential clinical relevance. We precautionarily suggest that dedicated trials should be performed in each study when merging different data sources prior to analyses.

Keywords:

Human fibula; Dual-energy CT scans; High-resolution surface scanning; 3D Geometric morphometrics comparability

1. Introduction

Several digitizing techniques, such as laser scanning, digital three-dimensional (3D) photogrammetry, computed tomography (CT) and micro-CT scanning, are widely implemented in morphological analyses, to evaluate bone shape and metric features. Such approaches consist of effective, repeatable, non-invasive diagnostic tools that allow the digitized object to be thoroughly examined without being physically manipulated, both in their external and internal geometry (Mantini and Ripani, 2009; Profico *et al.*, 2019; Uldin, 2017; Weber and Bookstein, 2011; Weber, 2001; 2014; 2015).

Often, the digitized 3D surface meshes obtained by CT and laser scanner are interchangeably adopted together and merged in morphological analyses (e.g., Frelat *et al.*, 2017; Mounier & Lahr, 2019; Sorrentino *et al.*, 2021, 2020a). Several studies have compared surface meshes obtained through 3D surface scanning with meshes obtained through medical CT scanning (Adams *et al.*, 2015; Brzobohatá *et al.*, 2012; Choi *et al.*, 2002; DeVries *et al.*, 2008; Farhni *et al.*, 2017; Fourie *et al.*, 2011; Lalone *et al.*, 2015; Ramme *et al.*, 2009; Waltenberger *et al.*, 2021) or micro-CT scanning (Teeter *et al.*, 2012) finding consistency among methods in measurement accuracy. Indeed, Adams and colleagues (2015) attested 0.4 mm mean surface deviations among surface meshes of hominin fossils digitized with a laser scanner, clinical CT and micro-CT scans. More recently, Waltenberger and colleagues (2021) compared surface meshes of human pelvises generated utilizing different digitizing methodologies (CT scans, laser scans, photogrammetry, MicroScribe digitizer) through a topological and landmark-based geometric morphometric (GM) approach. In their work, the average deviation among the surface meshes spanned between 100 to 200 μm , and the GM results attested the comparability among 3D meshes acquired with CT scanning, 3D structured light scanning and photogrammetry (Waltenberger *et al.*, 2021).

While most studies used the skull to investigate differences among scanning devices (e.g., Brzobohatá *et al.*, 2012; Farhni *et al.*, 2017; Pinsky *et al.*, 2006), others examined articular surfaces of long bones, revealing less than 0.4 mm average deviation among acquired elbow meshes (Lalone *et al.*, 2015). Similar values were obtained when assessing mesh accuracy of the bones of the

forearm (Oka *et al.*, 2009). Considering the human femur, topological analyses on CT- and laser scanner-generated meshes found a deviation spanning 0.71–0.79 mm (Gelaude *et al.*, 2008; Soodmand *et al.*, 2018; Stephen *et al.*, 2020). Additionally, Stephen and co-workers (2020) analyzed also the tibia and found comparable deviation of CT vs. laser scanner generated meshes (less than 0.71mm for tibiae).

Besides the scanning devices, other factors such as the choice of segmentation protocols may heavily influence the final outcome of mesh reconstructions, with potential impact on morphometric studies. Previous studies have tested the effect of bone segmentation procedures on mesh accuracy and comparability in morphometric analyses, including visual -based segmentation protocol, canny edge detection and other automated algorithms (Toro-Ibacache, 2013; Gunz, 2012; Fourie *et al.*, 2012; Engelbrecht *et al.*, 2013; Rathnayaka *et al.*, 2011; Ito, 2019). In particular, Ito (2019) found that single thresholding is often not suitable for a complex object with heterogeneous gray-value distributions and had statistically significant effect on shape and size variation.

Furthermore, the choice of smoothing procedures and the number of the iterations used have also shown possible effect on capturing anatomical information of virtually acquired bones, with potential impact on morphometric analyses (Profico *et al.*, 2016; Veneziano *et al.*, 2018).

The present work will explore the impact of different scanning devices, segmentation protocols and smoothing procedures on the reconstruction of surface models to test their comparability in morphometric analyses, with the aim of providing new data on the comparison of digitizing methodologies of long bones. Specifically, we test the accuracy and comparability of CT- and laser scanner-generated meshes in a sample of 13 modern human fibulae, considering the whole bone surface but also focusing on the extremities. The fibula has so far not been included in previous mesh accuracy assessments, despite its digitization in several biomechanical studies, alongside the tibia (e.g., Marchi, 2005; 2007; 2015b; Marchi, 2015a; Marchi *et al.*, 2019; Marchi *et al.*, 2021). In addition, fibular 3D meshes are routinely utilized in reconstructive surgery, in the assessment of tibio-fibular syndesmot

reductions performed after ankle fractures (e.g., Ebinger *et al.*, 2013; Souleiman *et al.*, 2021) and surgical planning of mandibular reconstructions from fibular free flaps (e.g., Damecourt *et al.*, 2020; Ren *et al.*, 2018; Ni *et al.*, 2021).

We also aim to provide data on the whole virtual acquisition pipeline, by testing the impact of segmentation protocols (i.e., half-maximum height and MIA-clustering protocols), smoothing procedures (i.e., Laplacian and Taubin smoothing) and 3D landmark configuration repeatability in inter-method comparisons. The results will add to our understanding of the use of 3D surface models obtained through different scanning techniques and procedures in 3D morphometric studies, with potential application in corrective surgical planning.

2. Materials and methods

2.1. The sample

The analyzed sample includes 13 left fibulae belonging to individuals from the Human Identified Skeletal Collection of Sassari, housed at the University of Bologna (Italy) and dating to the 19th- 20th century. The fibulae were selected for their general good state of preservation, except for a case (SS23) intentionally selected for the presence of a small areas of cortical damage, to test the surface reconstruction performance in the case of a partially damaged bone. The human sample includes 8 males and 5 females spanning 20–45-year-old.

2.2. Data acquisition, segmentation protocols, and smoothing procedures

2.2.1 Laser scanner acquisition

The sample of fibulae was scanned with ARTEC Space Spider 3D (Luxembourg), housed at the BONES Lab in the Department of Cultural Heritage of the University of Bologna. The ARTEC Space Spider 3D is a mobile and structured light laser scanner based on blue light technology, which uses trigonometric triangulation to calculate the distance among the points on the surface of the object and creates its relative point cloud. This machine provides a point-accuracy of 0.05 mm and mesh resolution of 0.1 mm. Moreover, it acquires both geometry and texture data (1.3 megapixel, 24-bits per pixel). The acquisition took place in two recording sessions for each specimen. The first

scan was performed by holding the device approximately perpendicular to the surface of the fibular diaphysis, with one of the extremities that was in turn placed vertically into a polystyrene support with a cavity in the center that accommodates the epiphysis, and by rotating the turning table. The non-supported epiphysis and part of the diaphysis were then acquired and subsequently a second scan was performed by inverting the fibular extremities. The integrated scanner software (Artec Studio 9) was utilized to merge the two sets of acquisitions, which were first cleaned and then manually aligned. These roughly aligned scans were then globally registered, with outlying points removed, and subsequently, a single polygonal 3D mesh is created with the Sharp Fusion algorithm, finally generating the 3D surface model that was then saved in .stl (Little Endian) format, following the procedures that are presented by the manufacturer user documentation (Artec Group, 2013).

2.2.1. CT scan acquisition

All fibulae were also digitized through computed tomography (CT), utilizing a Revolution Discovery CT dual energy, with GSI Revolution and HD Revolution configurations, housed at Istituto Ortopedico Rizzoli (Bologna, Italy). The acquisition protocol chosen was Extremity GSI. Once the minimum Field of View (FOV) had been achieved to optimize the best resolution, an acquisition with polychromatic beam at 100 kV and 360 mA was performed with slice thickness and acquisition interval at 0.625 mm. A “Standard” reconstruction filter with WW 400 and WL 40 was selected. Then, two reconstructions with monochromatic beams were made. The first reconstruction is at 70 keV with “Detail” reconstruction filter, with superior detail of bone acquisition in respect to the “Bone” filter with WW 2000 and WL 350. The second reconstruction is at 40 keV, always using the “Detail” filter with WW 400 and WL 400. The obtained reconstructed DICOM (16-bit grayscale, signed, voxel size ranging from 0.39 to 0.507 x 0.625) images at 40 keV, chosen for subsequent analyses, were then processed with Avizo 9.2 (Thermo Fisher Scientific) for image segmentation.

2.2.3. Image segmentations

On the CT-generated meshes, we applied the half-maximum height (HMH)

protocol outlined by Spoor and coworkers (1993), following the modified version detailed in Coleman and Colbert (2007), which included calculating the HMH for a row of pixels on the bone-to-air transition for 10 randomly selected slices, then averaged and applied to the whole stack. In addition, we also opted to visually segment the image stack utilizing a visual single-threshold based technique including voxels above -550, -600, -650 and -700 grayscale value intensity thresholds, to evaluate how possible differences of the chosen segmentation procedure influenced the 3D reconstruction overall quality, compared to the same surface data obtained by laser scanner. For both HMH and visual single-threshold based segmentations, thresholding was performed in Avizo 9.2 (Thermo Fisher Scientific, Waltham), determining grayscale intensity of selected voxels with either “histogram” or “magic wand” tools in the segmentation editor.

Moreover, we tested an additional segmentation protocol based on a machine-learning approach, MIA-clustering, which implements a clustering algorithm sorting the voxels of an image into clusters. This approach evaluates a global c-means clustering, and then separates the image into overlapping regions where more c-means iterations perform this sorting both globally and locally (Dunmore *et al.*, 2018). For our sample, we tested different numbers of clusters (from 3 to 5) at a 2% threshold and selected for subsequent analysis the image segmented utilizing a grid size of 3 pixels and 4 clusters, which allowed us to better represent intensity inhomogeneity of the input CT- scans images.

Lastly, an isosurface (.stl in Little Endian format) was generated for each segmentation protocol.

2.2.4. Smoothing procedures

Several smoothing procedures were tested on one specimen by calculating distances from the reconstructed CT-generated mesh and post-processed with different smoothing procedures to the homologous high-resolution laser scanner-generated mesh. Prior to smoothing testing, CT-generated meshes were segmented with the same segmentation protocol (HMH), chosen arbitrarily to reduce possible variation induced by different segmentation protocols and to control only for smoothing differences. The smoothing

procedures tested are Laplacian smoothing with or without surface preservation and Taubin smoothing (1995) with different number iterations (0.5; 1; 3; 5), generated in Meshlab 2020.12. Distances from CT-generated meshes smoothed with different procedures to the laser scanner-generated mesh were calculated, prior to alignment, following the procedures detailed below for all meshes comparisons.

2.3. Surfaces comparisons

The comparison among 3D surface meshes was performed in R v. 4.0.3. (R Core Team, 2020). Both CT- and laser scanner-generated meshes were uploaded and aligned by their Principal Axes with the function `pcAlign()` from the package “Morpho” (Schlager, 2017), using the CT-generated mesh as target for the alignment of the laser scanner-generated mesh, with an optimization procedure that minimizes root mean square errors (RMSE) between reference and target mesh, by testing all possible axes alignments with a rigid iterative closest point (ICP) procedure. Both iterations and subsampled points were set at 200, to compute the optimization procedure.

Topological analysis of distances among vertices of the CT- and laser scanner-generated meshes was then implemented by the function `meshDist()` from the package “Morpho” (Schlager, 2017), with the aligned laser-scanner generated mesh as the reference and the CT-generated mesh as the target. Mesh vertices distances were then represented by a polychrome scale with values ranging from 2.50 to -2.50 mm. Distances up to 0.20 mm were depicted in green and considered not relevant. Descriptive statistics (mean, standard deviation, and maximum displacement of surface scan mesh surface from the CT mesh) of distance among the vertices of the triangles that form meshes, were also calculated. We also provide %variation among CT- and surface scanner-generated meshes, calculated by dividing mean variation by maximum diameter at fibular midshaft multiplied by 100 (Table S1).

2.4. Geometric morphometric analysis

In addition to topological analysis, we assessed geometric distances between CT- and surface scanner-generated meshes by adopting a geometric morphometric approach based on fixed landmarks and curves and surface semilandmarks, specifically designed for evaluating the accuracy of the two

meshes at epiphyseal areas. Procrustes distances between landmarked surfaces generated by laser scanner and those generated by a CT-scanner were analysed. For each specimen, the laser scanner-generated meshes were compared to CT-scan generated meshes, which were segmented by MIA-clustering and HMH protocols separately. Additionally, a second digitization of the surface scanner-generated mesh was included in this analysis, to quantify possible intra-observer error due to template repeatability and not to due to scanning device.

A template of 142 landmarks and semilandmarks (Table 1 and Figure 1), modified from a previous version published by Marchi and colleagues (2022), was applied to targets (both CT- and laser scanner-generated models) utilizing Viewbox 4 software (dHal Software). Curve and surface semilandmarks were allowed to slide along the curves/surfaces to minimize the thin-plate spline (TPS) bending energy between the target and the template obtaining geometrically homologous semilandmarks (Slice, 2005; Gunz *et al.*, 2013). The set of semilandmarks for the proximal epiphysis (N = 94) and for the distal epiphysis (N = 48) were separated in order to perform two separate Generalized Procrustes Analysis (GPA) using the R package “geomorph” version 3.0.7 (Adams *et al.*, 2018). As a result, the semilandmark coordinates were superimposed with scale, position and orientation standardized, with semilandmarks being allowed to slide with each recursive update of the Procrustes consensus (Gunz *et al.*, 2005; Mitteroecker & Gunz, 2009; Rohlf & Slice, 1990; Slice, 2005; Sorrentino *et al.*, 2020a; 2020b; 2021).

Procrustes coordinates for each fibular epiphysis were then analyzed by Principal Component Analysis (PCA) to explore shape variations among specimens, differentiated by acquisition methodology, including for each individual the CT-generated meshes segmented with both HMH and MIA-clustering protocols, the surface scanner-generated mesh and a second landmark digitalization replica of the latter. Procrustes ANOVA was then adopted to test shape differences among scanning devices, utilizing Procrustes distances among specimens digitized with different scanning source, with a residual randomization procedure (RRPP = T, iterations = 1’000), with the R package “geomorph” version 3.0.7 (Adams *et al.*, 2018). Initially, we performed

a Procrustes ANOVA on shape distances among all specimens and its interaction with relative scanning devices in the model formula (shape ~ device), considering CT-generated meshes segmented with HMH and MIA-clustering protocols as a group and both laser scanner replicates as another group (i.e., CT vs. LASER). Additionally, to exclude the influence of intra-observer error on shape distances due to template repeatability and not solely to surface acquisition and segmentation method, a second Procrustes ANOVA (shape ~ method) was computed, this time separating CT-generated meshes segmented with HMH and MIA-clustering protocols and laser scanner replicates separately (i.e., CT-HMH vs. CT-MIA vs. LASER vs. LASER REPLICA).

Furthermore, a Post-hoc test was calculated for the latter Procrustes ANOVA, to evaluate the interaction among CT-generated meshes segmented with HMH and MIA-clustering protocols and laser scanner replicates, with pairwise function from the RRPP package (Collyer *et al.*, 2015; Collyer and Adams, 2018). This procedure controls for Type I errors in performing multiple tests, as the RRPP method performs the exact same random placement of residuals for every test statistic calculated, allowing us to consider these pairwise comparisons as separate inferences from the same test (Collyer *et al.*, 2015). The replicate design allowed us to assess whether variation due to scanning methods could be proxy of operator error (i.e., intra-observer error), therefore denouncing similar (possibly negligible) approximation. Finally, size comparisons among CT-generated meshes segmented with HMH and MIA-clustering protocols and the two laser scanner replicates were calculated using the centroid size, which is the square root of the summed squared distances between each semilandmark and the centroid of the semilandmark configuration (Slice, 2005). Centroid size distributions were evaluated visually by box-plots and differences among CT-generated meshes segmented with HMH and MIA-clustering protocols and laser scanner replicates were quantified by one-way ANOVA.

Landmarks and semilandmarks coordinates and sample list used to describe the specimens of the study are available (DOI: 10.5281/zenodo.6425379).

3. Results

3.1. Smoothing procedures

Consistency among smoothing procedures was revealed, but better results were offered when Laplacian smoothing with surface preserve was applied (Table 2; Figure S1). Therefore, for further analysis we opted to follow the application of a Laplacian smoothing with surface preserve at 0.5 iteration, minimizing post-processing procedures, to all CT-generated meshes.

3.2 Topological and visual meshes comparison

Descriptive statistics for topological distances among every CT-generated meshes segmented at different grayscale threshold values and with HMH and MIA-clustering protocols, and laser scanner-generated meshes are presented in Table 3. Topological differences among the CT-generated meshes with different segmentations and the laser scanner-generated meshes are presented in Figure 2. Comprehensively, the mean deviation between CT-generated meshes and laser scanner-generated meshes, considering all individuals at different segmentation protocols, vary between 0.35 mm and 0.70 mm, while maximum deviation values vary between 1.23 - 4.16 mm (Table 3).

For each individual, some degree of variation is seen among all its CT-based meshes generated from different segmentations. Individually, the range of variation of the deviation between CT-generated meshes and surface scanner-generated meshes measured for different segmentation protocols was between 0.09-0.24 mm, and for maximum deviation values was between 0.16-2.54 mm (Table 3). While regions of variations (i.e., location on bone surface) within each individual accounted by different segmentation protocols are quite subtle, both CT-generated meshes segmented with HMH and MIA-clustering protocols seem to better represent a larger portion of the diaphyses and epiphyseal areas, compared to other segmentation (-550, -600, -650 and -700 grayscale value intensity thresholds) (Figure 2, Figures S2-S12).

For specimen SS23 (Figure 3), taken singularly as an example due to the presence of a small cortical defect, we observe a more evident difference in regional distribution of variations accounted by segmentation protocol, located on the antero-medial portion of the fibular head, the styloid process,

the proximal tibio-fibular articular facet, the distal half of the interosseous border and the medial surface, the area of attachment of the interosseous ligament and the malleolar fossa. The small area of cortical loss at proximal epiphysis exceeded the range of mesh deviations in the first two segmentations and is thus mostly colored in white (Figure 3, a-c), but is captured by the last three protocols (Figure 3, d-f).

Indeed, for all specimens the CT-generated mesh is optimized when the MIA-clustering segmentation protocol is adopted, followed by HMH (Table 2). Deviation between CT-generated meshes segmented with MIA-clustering protocol and laser scanner-generated ones ranges between 0.35 and 0.56 mm of mean, while maximum deviation values are within a range of 1.27 and 2.99 mm. Overall, the average of the mean deviation among CT-generated meshes that were segmented with MIA-clustering protocol and laser scanner-generated ones is 0.42 mm, while the average of maximum deviations is 1.78 mm.

Indeed, taking as reference specimen SS163, considered representative of the variations seen in all specimens (Figure 4) for both HMH and MIA-clustering protocols there are areas with displacements from 0.2 to -0.2 mm, visible in green, in most of the posterior surface fibular diaphysis, while anterior and medial surfaces are slightly more displaced than the former, staying however within maximum 0.50 mm distance. The lowest divergence between CT scan and laser scanners is observed at the level of epiphyses, at the proximal tibio-fibular articular facet and the distal fibular-talar articular facet, whereas some degree of displacement is found around the fibular head, and at the superior border of the proximal fibular-talar articular facet.

3.2. 3D GM analysis for meshes comparison and intra-observer error

The PCA plots of the shape coordinates for the proximal and distal epiphysis show that the CT-generated specimens segmented with both HMH and MIA-clustering protocols either overlapped or clustered in the very proximity of the homologous individual digitized with laser scanner and its replica (Figure 5; Figure S13-S14). Results are corroborated by the Procrustes ANOVA for both distal and proximal epiphysis (Table 4) comparing shape variation due to scanning device considering CT-generated meshes (pooled meshes segmented

with HMH and MIA protocols) and laser scanner (original and replicates combined together), with no significant differences found. Considering both proximal and distal epiphyses, only 1.38 – 1.43 % of variance (R^2) is explained by differences in scanning device, while 98.57 - 98.62% of variance is explained by residuals, suggesting that biological variations may account for the large proportion of this variance, independently from the scanning devices.

Moreover, the PCA plots for the proximal and distal epiphysis show that the replicates (digitized on the laser scanner meshes) maintain similar variances and plot closely to their homologous meshes coming from the first digitization on laser scanner. (Figure 5). Indeed, when Procrustes ANOVA is computed considering CT-generated meshes segmented with HMH and MIA-clustering protocols and laser scanner replicates separately (Table 5), again no significant difference among all configurations emerged ($p > 0.05$ for both epiphyses). The Procrustes distance between LASER vs. LASER REPLICA is actually lower than distances among all other comparisons (Table 6), ultimately denoting low intra-observer error in the application of the template. This result likely indicates that (1) the intra-observer error is negligible, (2) and that the inter-method comparisons are not influenced by repeating the template on the same individual (intra-observer error)

Centroid size differences among CT-generated meshes segmented with HMH and MIA-clustering protocols and laser scanner replicates are appreciable but statistically not significant for both epiphyses (Figure 6). Overall, CT-generated meshes either segmented with HMH and MIA-clustering protocols tend to slightly overestimate mesh size, but ANOVA test confirm that for both epiphyses comparisons are not significant ($p > 0.05$).

4. Discussion and conclusion

Both topological and geometric morphometric analysis demonstrated that CT scanning and laser scanning provide similar meshes of the fibula, fully comparable to one another, suggesting these acquisition devices could be adopted interchangeably in morphological analyses. Deviations among the meshes obtained by using the two devices are comparable with those reported in previous studies (Brzobohatá *et al.*, 2012; Farhni *et al.*, 2017; Lalone *et al.*, 2015; Waltenberger *et al.*, 2021). Our results agree with those obtained on

long bones by Soodmand and co-workers (2018), adopting a similar acquisition protocol: they found a mean root mean square error below 0.84 mm between CT and laser-scanning meshes and reported consistent inter- observer agreement among processing of different laboratories acquiring and elaborating the same model. Our data are also consistent with those of Stephen and colleagues (2020), who examined a sample of long bones, which despite some differences in the CT scanning protocols in relation to slice thickness (1 mm instead of our 0.65 mm), found similar mean deviation values between CT and laser-scanning meshes (tibia: 0.71-0.75 mm; femur: 0.69-0.80 mm), with no significant difference between surface accuracy and methodology. Our results on the fibula fall within this accuracy range, spanning an average of 0.35-0.56 mm when CT-obtained meshes segmented with MIA-clustering protocol are considered.

The comparison of our results to those of previous studies on comparability of the surface reconstructions (Brzobohatá *et al.*, 2012; Choi *et al.*, 2002; DeVries *et al.*, 2008; Farhni *et al.*, 2017; Ramme *et al.*, 2009) should account for differences in protocols of acquiring and elaborating volumetric data, and the variety of the examined anatomic components (skull, phalanges). For instance, Brzobohatá *et al.* (2012), Choi *et al.* (2002), DeVries *et al.* (2008), Ramme *et al.* (2009) utilized a slice thickness of 1.0 mm, within their specific image acquisition parameters on medical CT scans. DeVries *et al.*, (2008) revealed that the overall mean difference between the manually segmented CT models of hand phalanxes and their laser surface scan was 0.20 mm, with minimal effect of different smoothing procedures on surface representations, also coherent with our results (Table 1; Figure S1).

Concerning the smoothing procedures, our results highlight only minimal variation related to procedure choice (Table 2) in agreement with a previous work on smoothing procedures that have showed that smoothing algorithm affected mesh topology differently, despite retaining measurement accuracy in most reconstructions (Veneziano *et al.*, 2018). While Veneziano and colleagues (2018) found that the Taubin algorithm ensured avoiding information loss on the surface of the mesh, we found a lower mean distance between the CT-generated mesh smoothed with Laplacian algorithm with

surface preserve. It is important to notice, though, that in our testing the best results were obtained when the iterations were minimal.

Regarding the segmentation protocols, our results indicate that different chosen single-thresholding value influences topological differences of CT scan and laser scanner meshes, suggesting the necessity of a homogeneous segmentation protocol. Previous works (Engelbrecht *et al.*, 2013; Fourie *et al.*, 2012; Rathnayaka *et al.*, 2011) found that, despite differences in accuracy of the 3D model in relation the segmentation methods, even the least accurate of the analyzed segmentation protocols (the single-threshold technique) produced comparable 3D reconstruction, minimally deviating from their high-resolution gold standard (0.18–0.24 mm). This is consistent with our study, where the adoption of a single-threshold segmentation protocol provided average deviations mostly below the maximum resolution of the CT scan of 0.625 mm in slice thickness, even in cases of small cortical defects, despite with a less accurate performance. On the other hand, MIA-clustering and HMH protocols offered non-relevant deviations (-0.02 - 0.2 mm) at epiphyses and in larger portions of the diaphysis (Figure 3), compared to meshes generated with other segmentations based on single grayscale values, possibly reflecting shortcomings of single grayscale value thresholding in detecting thinner cortical feature on the bone surface. Indeed, Ito (2019) found that single grayscale value thresholding, including HMH protocol, might induce excessive erosion in 3D reconstructions, especially in low-density areas, suggesting caution when combining segmentation protocols within a dataset for detailed GM analysis (e.g., asymmetry), since inter-method error may be greater than shape variations explained by asymmetry. In fact, single thresholding segmentations may induce errors due to intensity inhomogeneity of CT data, and therefore may not capture fine bone surface features (Scherf & Tilgner, 2009). Adopting a segmentation protocol such as MIA-clustering, based on a machine-learning approach that adopts a global and local fuzzy c-means clustering, here implemented on medical CT images, yielded better results than single-thresholding segmentations, providing a practical, inexpensive solution to possible low resolution and grayscale intensity shortcomings (Dunmore *et al.*, 2018). This segmentation approach could also be useful in

clinical applications, for the 3D evaluation of the tibio-fibular syndesmotomic space, as it is a timesaving technique (Ebinger *et al.*, 2013). Such variations may also have implications for GM analysis, as landmarks situated on sharp peaks may be sensitive to volume-averaging effect, causing blurring in bone regions with low density or in adjacent bones with adjacent soft tissue (Wang, Greenspan and Ellis, 2006).

In our results, we found that our landmark and semilandmark configurations mostly overlap in homologous individuals scanned with CT and laser scanner (Figure 5) and only 1.38 – 1.43 % of total variance in the dataset is explained by difference in scanning device, with no statistically significant differences in morphology between the two methods. The results of our PCA analyses (Figure 5) are comparable to those of Marcy and coworkers (2018) and Waltenberger *et al.*, (2021), even when both sets of scanning digitization are added, with each individual, either CT- or laser scanned, clustering together. However, while Waltenberger *et al.*, (2021) found no significant difference in their Procrustes ANOVA in accordance with our own results, Marcy and coworkers (2018), who compared laser scanner and micro-CT derived mesh reconstructions of mice skulls, found that the percentage of variance explained by asymmetry (directional and fluctuating) was lower than the percentage of variance explained by scanning device. According to the authors, this would suggest that analyses of asymmetry with a combination of scanning methods scans may be subject to systematic error. In both ours and Waltenberger *et al.* (2021) cases, it is possible that the relatively big size of the fibula in contrast to the diminutive size of mice skulls may have prevented this effect, therefore supporting the comparability of scanning methodology through geometric morphometric methods and the combination of different datasets in GM analysis of human bone elements. Moreover, as in our sample 98.57 - 98.62% of variance is explained by factors other than scanning methodology, it is possible to infer that such shape variations may account for biological diversity within our sample.

Centroid size comparisons for both epiphyses revealed that, while CT-generated meshes are larger than laser scanner-generated meshes, their size is comparable. The larger centroid size of CT-generated meshes also confirms

the observations of Table 3, with mean distances between CT-generated meshes and laser scanner-generated meshes indicating all positive values, suggesting that the latter meshes possess indeed smaller surface and areas than the formers.

In our analysis, inter-method comparisons showed good reproducibility of landmark digitization, with lower Procrustes distance between each set of CT and laser scanner replicates than inter-methods, suggesting low intra-observer error. This is consistent with Fruciano and co-workers (2017), who found that when combining landmark data from multiple devices and digitized by multiple operators and test for the presence of bias, a larger amount of variance was explained by the operator compared to the device. The authors however also found that, similar to Robinson and Terhune (2017), inter-method and inter-observer errors may be greater than small intraspecific or closely related species shape variations. Indeed, the authors advocate for testing accuracy trials of different methods and comparability of observers prior to combination of different data source.

In conclusion, we validated the comparability and combination of 3D reconstruction of human fibulae, generated by dual-energy CT scan and two sets of 3D laser scanner replicates, confirming substantial topological similarity of the meshes reconstructed with the two methods and corroborating their interchangeability in GM studies, as no significant inter-method and intra-observer difference is found in shape analysis, with potential applications in clinical context (Souleiman *et al.*, 2021). A possible limitation of the present study is the use of laser scanner-generated meshes as the gold standard comparison from which distances of CT-generated meshes are calculated, assuming their closeness to the real bone surface as being more accurate. In our analyses, in fact, we did not evaluate this proximity to the real specimens, but on the basis of previous evaluations utilizing the same laser scanner employed here (Adams *et al.*, 2015), and given that the 3D accuracy stated by the manufacturer is to 50 μm , we could indeed infer that the laser scanner surfaces are the most accurate approximations. We suggest, in accordance with previous studies (Marcy and coworkers, 2018; Robinson and Terhune, 2017; Waltenberger *et al.*, 2021), that prior to formal analysis, coherence of

both CT and laser scanning protocols, including assessment of scanning settings and resolutions, segmentations and reconstruction post-processing, should be thoroughly evaluated, in case of merging different data sources, possibly with dedicated trials.

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Data availability statement

All data acquired and analyzed in this work is available upon request addressed to the corresponding author. Landmarks coordinates and sample lists for geometric morphometric analysis are available (DOI: 10.5281/zenodo.6425379).

Conflict of interest

The authors declare no potential conflict of interest.

Authors' contributions

AP conceptualization, investigation, formal analysis, validation, writing-original draft, methodology, writing-review, and editing; RS validation, writing-original draft, methodology, writing-review, and editing; VN data acquisition, formal analysis, validation; SD conceptualization, supervision of the research, methodology, data acquisition, validation, software; DM, SB, MGB conceptualization, supervision of the research, methodology, project administration, software, writing-review and editing.

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Tables

Landmarks	Definition
L1	Point where the <i>fibular anterolateral border</i> divides into two ridges: the proximal apex of the <i>subcutaneous triangular surface</i> (STS)
L2	Most medial point of the medial border of the STS
L3	Most lateral point of the lateral border of the STS
L4	Most distal point of the <i>lateral malleolus</i> in anterior view
L5	Most distal point of the posterior border of the <i>malleolar fossa</i>
L7	Most anterior point on the anterior border of the <i>proximal fibular-talar articular facet</i> (PAF)
L8	Point between the anterior border of PAF and the <i>anterior border of distal fibular-talar articular facet</i> (DAF)
L9	Most distal point of DAF
L10	Most proximal point on the posterior border of DAF
L12	Most posterior point of the proximal border of PAF
L13	Most proximal point of <i>proximal tibio-fibular articular facet</i>
L14	Most proximal point of <i>interosseous tibio-fibular ligament</i> (ILA) insertion
L15	Most proximal point on styloid process of fibular head in medial view
L16	Most antero-proximal point on anterior border in medial view (above fibular neck)
L17	Most postero-proximal point on posteromedial border in medial view (above fibular neck)
L18	Most postero-proximal point on posterior border in lateral view (above fibular neck)

Curves	Definition	Number of semi-landmarks
C_1->2	Medial border of the <i>subcutaneous triangular surface</i> (STS)	5
C_1->3	Posterior border of the STS	7
C_7->8	Anterior border of the <i>proximal fibular-talar articular facet</i> (PAF)	1
C_8->9	Anterior border of the <i>distal fibular-talar articular facet</i> (DAF)	1

C_9->10	Posterior border of the DAF	1
C_10->12	Posterior border of the PAF	1
C_8->10	Border between the PAF and DAF	1
C_13->13	Outline of <i>proximal tibio-fibular articular facet</i>	6
C_7->12	Proximal border of the PAF	2

Surfaces	Definition	Number of semi-landmarks
SSML_malleolar fossa	Surface of the <i>malleolar fossa</i> , attachment site of the <i>transverse tibiofibular</i> and <i>posterior talofibular ligaments</i> .	7
SSML_ILA	Attachment surface of <i>interosseous tibio-fibular ligament</i> and part of <i>interosseous membrane</i> (ILA)	13
SSML_fibular groove	Groove for tendons of m. <i>peroneus longus</i> and m. <i>tertius</i> and attachment site of <i>posterior tibiofibular ligament</i> .	13
SSML_STS	<i>Subcutaneous triangular surface</i> (STS)	24
SSML_FiTal1Ar	Proximal <i>fibular-talar articular facet</i> (PAF)	4
SSML_FiTal2Ar	Distal <i>fibular-talar articular facet</i> (DAF)	3
SSML_head	Proximal <i>tibio-fibular articular surface</i>	5
SSML_prox_ep	Surface of <i>proximal epiphysis</i>	32

Table 1. Fibular landmarks and (semi)landmarks identification, definition, and number.

<i>Laplacian smoothing with SP</i>	Mean (mm)	Sd (mm)	Max (mm)	Min (mm)
0.5	0.51	0.27	1.71	0
1	0.51	0.28	1.79	0
3	0.51	0.29	1.75	0
5	0.51	0.32	1.76	0
range	0.00	0.05	0.08	0.00
<i>Laplacian smoothing</i>				
0.5	0.52	0.29	1.73	0
1	0.52	0.31	1.97	0
3	0.55	0.25	1.97	0
5	0.52	0.27	2.1	0
range	0.03	0.06	0.37	0.00
<i>Taubin smoothing</i>				
0.5	0.51	0.28	1.80	0
1	0.52	0.28	1.77	0
3	0.53	0.28	1.78	0
5	0.53	0.31	1.94	0
range	0.02	0.03	0.17	0
<i>No smoothing</i>	0.51	0.27	1.71	0

Table 2. Descriptive statistics (mean. standard deviation. minimum and maximum values) of distances among vertexes of CT-generated mesh of SS163, segmented with HMM protocol, and subjected to different smoothing protocols, and its respective laser scanner- generated mesh. Smoothing protocols evaluated presence/absence of surface preserve filter and a different number of iterations (0.5, 1, 3, 5). SP= surface preserve.

	Mean (mm)	Sd (mm)	Max (mm)	%		Mean (mm)	Sd (mm)	Max (mm)	%
<i>SS4</i>					<i>SS95</i>				
-550	0.59	0.25	1.39	3.44	-550	0.48	0.32	3.33	3.45
-600	0.62	0.25	1.49	3.61	-600	0.52	0.33	3.52	3.74
-650	0.68	0.26	1.51	3.96	-650	0.56	0.37	3.81	4.03
-700	0.68	0.26	1.52	3.96	-700	0.6	0.38	3.7	4.32
MIA	0.56	0.2	1.57	3.26	MIA	0.4	0.31	2.99	2.88
HMH	0.56	0.22	1.5	3.26	HMH	0.46	0.31	3.04	3.31
range	0.12	0.06	0.18	0.7	range	0.2	0.07	0.82	1.41
<i>SS14</i>					<i>SS105</i>				
-550	0.48	0.3	4.06	3.38	-550	0.63	0.27	1.83	3.57
-600	0.51	0.27	3.3	3.59	-600	0.64	0.26	1.72	3.63
-650	0.54	0.29	2.57	3.81	-650	0.68	0.28	1.8	3.85
-700	0.59	0.29	2.46	4.16	-700	0.7	0.27	1.88	3.97
MIA	0.39	0.24	1.94	2.75	MIA	0.51	0.33	2.18	2.89
HMH	0.46	0.32	4.16	3.24	HMH	0.61	0.28	1.75	3.46
range	0.2	0.08	2.22	1.41	range	0.19	0.07	0.46	1.08
<i>SS23</i>					<i>SS115</i>				
-550	0.54	0.55	3.76	3.3	-550	0.55	0.2	1.23	3.44
-600	0.5	0.42	3.34	3.06	-600	0.58	0.21	1.33	3.63
-650	0.5	0.38	3.03	3.06	-650	0.6	0.2	1.25	3.75
-700	0.5	0.36	3.6	3.06	-700	0.63	0.2	1.55	3.94
MIA	0.45	0.29	2.26	2.75	MIA	0.4	0.16	1.27	2.5
HMH	0.47	0.41	3.62	2.87	HMH	0.54	0.22	1.39	3.38
range	0.09	0.26	1.5	0.55	range	0.23	0.06	0.32	1.62
<i>SS26</i>					<i>SS163</i>				
-550	0.59	0.27	2.04	3.5	-550	0.53	0.31	1.7	3.7
-600	0.61	0.27	1.88	3.61	-600	0.54	0.31	1.54	3.77
-650	0.64	0.26	2.01	3.79	-650	0.58	0.27	1.48	4.05
-700	0.66	0.27	2.01	3.91	-700	0.66	0.36	1.73	4.61
MIA	0.56	0.26	2.03	3.32	MIA	0.42	0.21	1.48	2.94
HMH	0.58	0.26	2.04	3.44	HMH	0.51	0.27	1.71	3.56
range	0.10	0.01	0.16	0.59	range	0.24	0.10	0.23	1.68
<i>SS28</i>					<i>SS215</i>				
-550	0.46	0.28	3.03	4.14	-550	0.58	0.22	1.66	4.19
-600	0.49	0.25	2.19	4.41	-600	0.61	0.24	1.92	4.41
-650	0.47	0.25	2.24	4.23	-650	0.63	0.27	2.11	4.56
-700	0.54	0.27	1.49	4.86	-700	0.67	0.33	2.9	4.84
MIA	0.39	0.23	1.63	3.51	MIA	0.45	0.22	1.86	3.25

HMH	0.45	0.26	2.93	4.05	HMH	0.57	0.26	2	4.12
range	0.15	0.05	1.54	1.35	range	0.22	0.11	1.24	1.55
SS48					SS224				
-550	0.44	0.22	3.32	3.06	-550	0.45	0.26	2.44	2.86
-600	0.45	0.2	2.99	3.13	-600	0.49	0.28	2.86	3.12
-650	0.48	0.2	2.75	3.34	-650	0.53	0.28	2.79	3.37
-700	0.54	0.23	2.37	3.76	-700	0.58	0.28	2.71	3.69
MIA	0.36	0.17	2.12	2.5	MIA	0.41	0.24	1.99	2.61
HMH	0.41	0.23	3.39	2.85	HMH	0.46	0.24	2.16	2.92
range	0.18	0.06	1.27	1.25	range	0.17	0.04	0.87	1.08
Average MIA	0.42	0.22	1.78	2.8	SS234				
Average HMH	0.45	0.27	2.29	3.02	-550	0.37	0.27	3.81	2.55
					-600	0.38	0.24	3.29	2.62
					-650	0.42	0.25	2.79	2.89
					-700	0.46	0.27	1.47	3.17
					MIA	0.35	0.21	1.27	2.41
					HMH	0.36	0.53	2.54	2.48
					range	0.11	0.32	2.54	0.76

Table 3. Descriptive statistics (mean, standard deviation, and maximum values) of distances calculated among CT- and laser scanner- generated meshes (vertexes) for the 13 specimens analysed. %Var is calculated as (mean distance/max diameter at midshaft*100). Maximum Midshaft diameters at midshaft for each specimen are presented in Table S1. For each specimen, segmentations at four different grayscale values are considered (-550, -600, -650, and -700; for SS163: -500, -550, -600, -650). For each specimen, total individual range variation in deviations considering all segmentation protocols is indicated at the bottom of each specimen's section. The total average of deviations considering every segmentation with Half Maximum Height (HMH) and MIA-clustering protocols for all specimen, are indicated in the last rows. Values are in mm.

Procrustes ANOVA						
Distal epiphysis						
Device	Df	SS	MS	Rsq	F	p value
Device	1	0.00605	0.0060516	0.01378	0.6986	ns
Residuals	50	0.43313	0.0086626	0.98622		
Total	51	0.43918				
Proximal epiphysis						
Device	Df	SS	MS	Rsq	F	p value
Device	1	0.01786	0.017857	0.0143	0.7253	ns
Residuals	50	1.23096	0.024619	0.9857		
Total	51	1.24881				

Table 4. Results of Procrustes ANOVA for distal and proximal epiphyses, considering computed tomography (CT)-generated meshes segmented with HMH and MIA-clustering protocols together and both laser scanner replicates together, comparing shape variation due to scanning device (i.e., CT vs. LASER).

Procrustes ANOVA						
Distal epiphysis						
Method	Df	SS	MS	Rsq	F	p value
Method	3	0.00807	0.0026917	0.01839	0.2997	ns
Residuals	48	0.43111	0.0089814	0.98161		
Total	51	0.43918				
Proximal epiphysis						
Method	Df	SS	MS	Rsq	F	p value
Method	3	0.02117	0.0070581	0.01696	0.276	ns
Residuals	48	1.22764	0.0255758	0.98304		
Total	51	1.24881				

Table 5. Results of Procrustes ANOVA for distal and proximal epiphyses, considering computed tomography (CT)-generated meshes segmented with HMH and MIA-clustering protocols and laser scanner replicates separately (i.e., CT-HMH vs. CT-MIA vs. LASER vs. LASER REPLICA)

Distal epiphysis					
	d	UCL (95%)	Z	p value	global p value
CT-HMH: CT-MIA	0.016164350	0.05585739	-2.608923	ns	ns
CT-HMH: Laser1	0.024059392	0.05637040	-1.038789	ns	
CT-HMH: Laser2	0.024081671	0.05640215	-1.042411	ns	
CT-MIA: Laser1	0.022243496	0.05902505	-1.353978	ns	
CT-MIA: Laser2	0.022798490	0.05591536	-1.278960	ns	
Laser1: Laser2	0.007071018	0.05844946	-6.105541	ns	
Proximal epiphysis					
	d	UCL (95%)	Z	p value	global p value
CT-HMH: CT-MIA	0.01782615	0.08976858	-4.264950	ns	ns
CT-HMH: Laser1	0.03426822	0.08563265	-1.721336	ns	
CT-HMH: Laser2	0.03956952	0.08771015	-1.360360	ns	
CT-MIA: Laser1	0.03783650	0.08875301	-1.517139	ns	
CT-MIA: Laser2	0.04281501	0.08964866	-1.073916	ns	
Laser1: Laser2	0.01387727	0.08989073	-4.294620	ns	

Table 6. Post-hoc Procrustes distances pairwise comparisons among computed tomography (CT)-generated meshes segmented with HMH and MIA-clustering protocols and two sets of laser scanner replicates considered separately. d=Procrustes distance; UCL = Upper Confidence Limit.

Figures

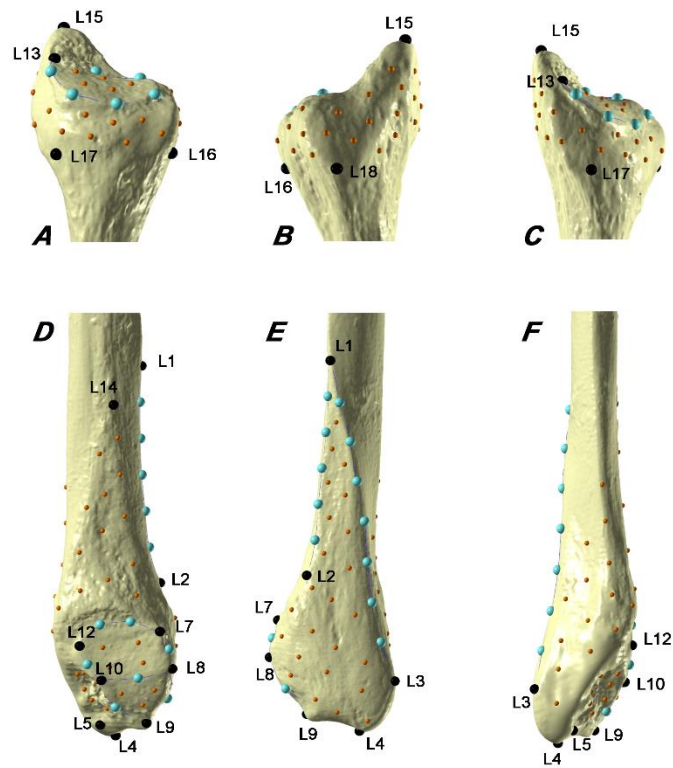


Figure 1. Landmarks (black), curve (light blue) and surface semilandmarks (orange) digitized on a left fibula (A, medial view, proximal; B, lateral view, proximal; C, postero-medial view, proximal; D, medial view, distal; E, lateral view, distal; F, posterior view, distal). See Table 1 for a detailed description of the anatomical landmarks.



Figure 2. Computed tomography (CT) -generated meshes for every segmentation protocol for a representative specimen (SS163), with relative distances (-2.5 to 2.5 mm, from violet to red; in white, areas where deviations exceed this range), from their laser-scanner generated equivalent. a, CT segmented at -550 grayscale intensity; b, CT segmented at -600 grayscale intensity; c, CT segmented at -650 grayscale intensity; d, CT segmented at -700 grayscale intensity; and e, CT segmented with Half Maximum Height protocol (HMH) and f, MIA-clustering protocol (MIA). Other specimens are represented in Figures S2-S14 in Supplementary Information.

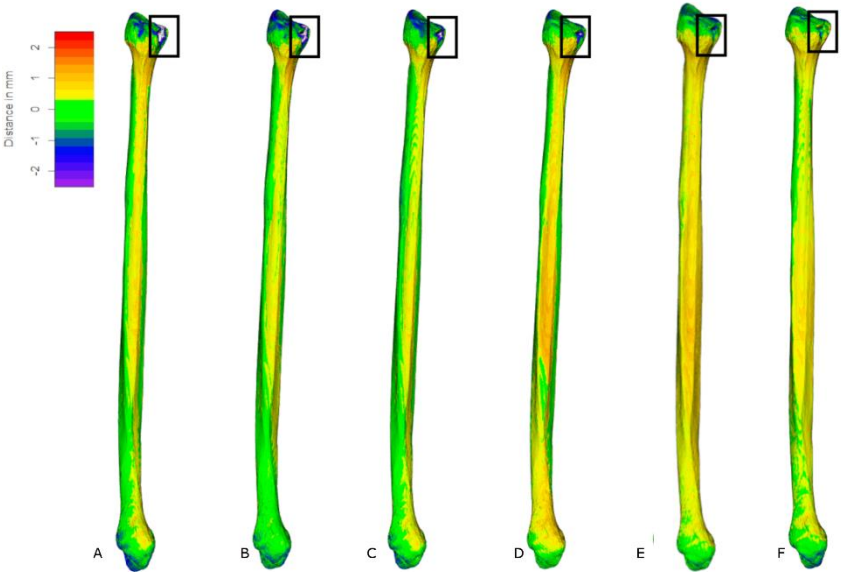


Figure 3. Computed tomography-generated meshes on one specimen (SS23, left fibula) segmented with every segmentation protocol with relative distances (-2.5 to 2.5 mm, from violet to red; in white, deviations exceed this range), from its laser-scanner generated equivalent; a, CT segmented at -550 grayscale intensity; b, CT segmented at -600 grayscale intensity ; c, CT segmented at -650 grayscale intensity ; d, CT segmented at -700 grayscale intensity ; and e, CT segmented with Half Maximum Height protocol (HMH) and f, MIA-clustering protocol (MIA). Note the small area of cortical defect (black square).

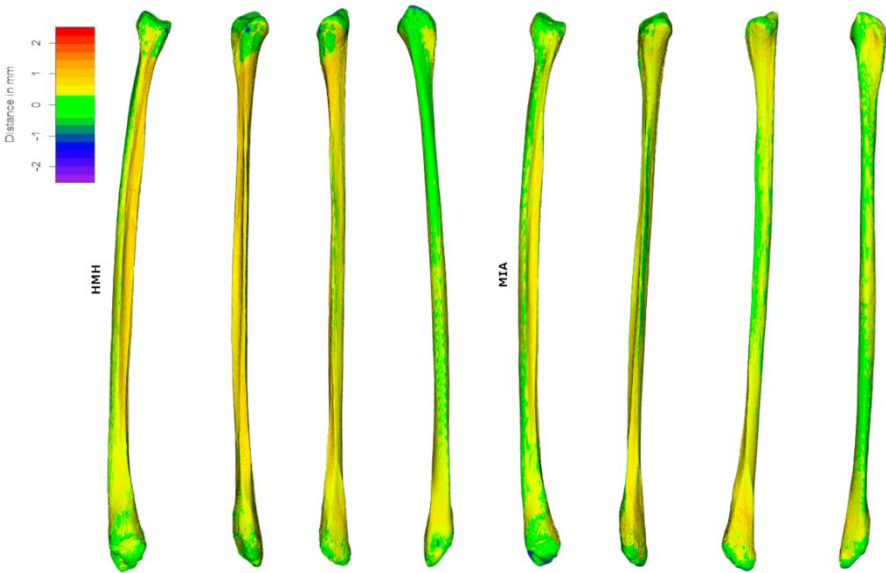


Figure 4. Computed tomography-generated meshes on one representative specimen (SS163, left fibula) segmented with Half Maximum Height protocol (HMH) and f, MIA-clustering protocol (MIA), with relative distances (-2.5 to 2.5 mm, from violet to red; in white, deviations exceed this range), from its laser-scanner generated equivalent, seen in medial, anterior lateral, and posterior view (from left to right).

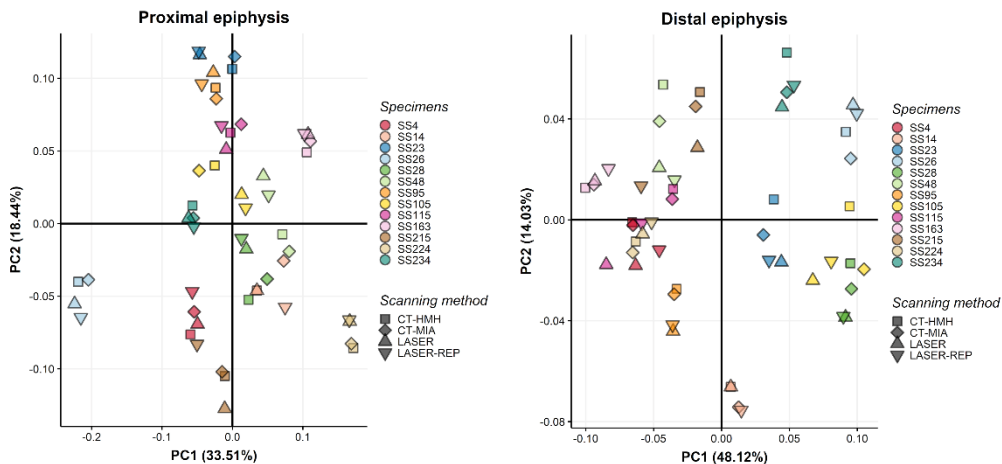


Figure 5. Principal component analysis plot considering landmark configurations applied to computed tomography (CT)-generated segmented with HMH (square), and with MIA-clustering (rhombus), and laser scanner-generated individuals and replicates (triangles) for the distal and the proximal epiphysis.

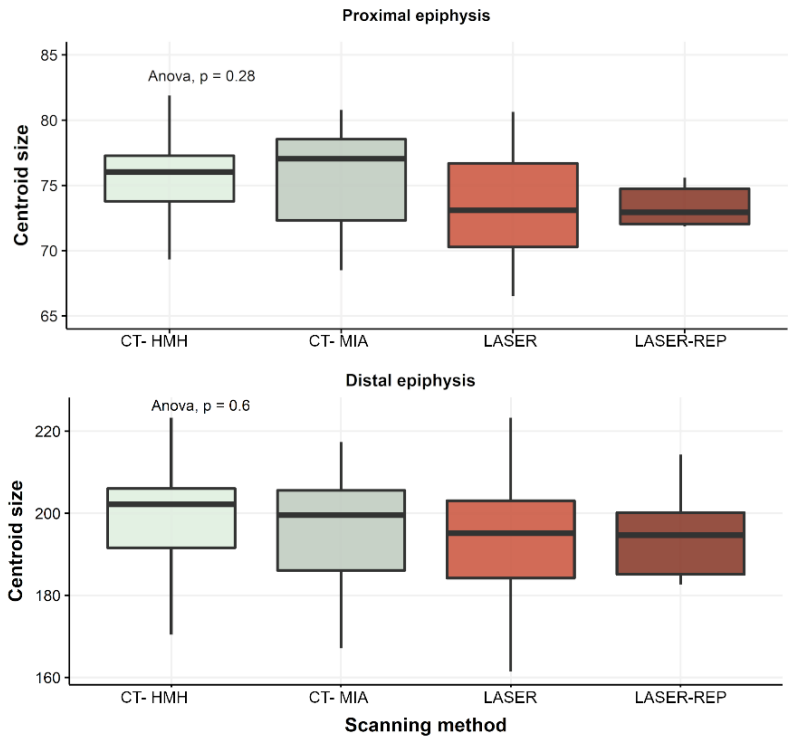


Figure 6. Centroid size comparisons among landmark configurations applied to computed tomography (CT)-generated segmented with HMH, with MIA-clustering (in gray), and laser scanner-generated individuals and replicates (in red), displayed for both proximal and distal epiphysis and results of one-way ANOVA.

Supplementary materials are available at: [joa13714-sup-0001-Supinfo.docx](#)



CHAPTER 3

Results

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INTRASPECIFIC
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PAPER I

Intraspecific variation: sexual dimorphism

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biology



Article

Sexual Dimorphism in the Fibular Extremities of Italians and South Africans of Identified Modern Human Skeletal Collections: A Geometric Morphometric Approach

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Title:

Sexual dimorphism in the fibular extremities of Italian and African identified modern human skeletal collections: a geometric morphometric approach

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Abstract

Fibular metric variations have revealed their potential in distinguishing between males and females, however the fibula remains scarcely analysed in studies of sexual dimorphism. Considering that fibular sex assessment may find applications in both forensic and bioarchaeological contexts, this work aims at investigating sexually dimorphic features in fibular proximal and distal epiphyses through geometric morphometrics. Three samples, for a total of 136 left fibulae, from two Italian (Emilia-Romagna, N = 47; Sardinia, N = 72) and one South African (Dart Skeletal Collection, N = 17) identified skeletal collections were virtually acquired through CT and laser scanning. A total of 142 (semi)landmarks were used to capture the shape of both proximal and

distal fibular epiphyses. After Generalize Procrustes superimposition, the Procrustes coordinates of the proximal and distal fibular epiphyses were used separately to assess variation due to sex. Linear Discriminant Analysis (LDA) with leave-one-out cross-validation testing was used to assess classification accuracy of sex based on shape and form space Principal Components (PCs), as well as centroid size.

Results show that fibular epiphyses are smaller in females than in males, with narrower articular surfaces in both extremities. No significant sexual dimorphism emerges in shape space for any sample, while relevant sexual differences emerge in fibular form and size for the two Italian samples but not for the South African one. Indeed, LDA on form PCs offers an accuracy above 80 %, when the samples are pooled, and reaches an accuracy of 80-93% when the Italian samples are considered separately. However, our method on form PCs was not successful for the South African sample (50-53% accuracy).

These results show relevant morphological variation in relation to fibular form and size, with a degree of accuracy that indicate the utility of the present method for sexing human fibulae in both forensic and bioarchaeological contexts.

Keywords:

sex determination; human fibula; epiphysis; 3D geometric morphometrics; leg bones.

1. Introduction

Shape and size of leg bones are widely used in both bioarchaeological and forensic contexts as sex indicators (e.g., Iscan & Miller-Shaivitz, 1984; Kranioti & Apostol, 2015; Brzobohatà *et al.*, 2016; Fasemore *et al.*, 2018). Generally, males display larger and more robust tibiae and fibulae, with more robust lower extremities than females (Brothwell, 1981; Ubelaker, 1989) due to environmental and hormonal contexts and physical load as well, that contribute to sexual variations of body shape and size/proportions (Ruff, 1987).

While the human tibial shape has been extensively studied with biomechanical and morphometric (both traditional and virtual) approaches for sex assessment (Kimura, 1971; Ruff & Hayes, 1983; Brzobohatà *et al.*, 2014; 2015; 2016;), few studies include the fibula. Sex assessment based on multivariate analyses of fibular measurements in populations with different ancestries have reported accuracies of 80–86% (Sacragi, Ikeda & Terada, 1993; Spradley & Jantz, 2011; Spradley, Anderson & Tise, 2015; Moore *et al.*, 2016; Krüger *et al.*, 2017). Such studies, however, focus solely on gross anatomical fibular features and linear measurements, such as maximum length and maximum diameters, not considering the shape of the extremities. The most often given reason as to why there are few studies focusing on the fibula is the scarcity of fibulae in both forensic and bioarchaeological contexts, with only 18-50% of preserved fibular segments out of the expected total (Waldron, 1987; Spenneman, 1992, Stojanowski, Seidemann & Doran, 2002). However, it has been reported that for fragments below 20% and above 80% of total bone length (i.e., distal and proximal fibular extremities, respectively) the percentage of preservation is between 57 - 90% and 61-91, respectively (Willey, Galloway & Snyder, 1997).

Singh and Singh (1976) have included in their analysis measurements of distal articular surfaces, suggesting that the width of the distal extremity, together with fibular length and mid-shaft circumference, correctly estimated sex with 99% of accuracy. In another study, Sacragi and Ikeda (1995) focused on the talar articular surface, the malleolar fossa, and the lateral malleolus, and using a population specific discriminate function, they were able to correctly classify males at 90.1% and females at 91.4%. Naidoo and colleagues (2015), in

addition, examined different linear and angular dimensions of the talar facet, and found significant variations among males and females, and confirmed consistency among different populations.

Several works examined measurements associated with fibular nutrient foramen in South African populations (Naidoo *et al.*, 2015; Mazenganya & Billings, 2016; Fasemore *et al.*, 2018). Fasemore and colleagues (2018) have found relevant sexual differences among measurements of diameters and circumference taken at the level of the fibular nutrient foramen, with a discriminatory cross-validated accuracy of 73% with combined measurements. Interestingly, the authors also highlighted how measurements of distance between the most proximal end of the fibula and nutrient foramen is not sexually dimorphic. The proximal end of the fibula is in fact rarely studied in anthropology for sex determination purposes, and only studies accounting for three-dimensional fibular shape variations included the proximal extremity, along other tibial and fibular regions, in their analysis (Zhang *et al.*, 2016; Tümer *et al.*, 2019; Nolte *et al.*, 2020; Audendart *et al.*, 2019; Bruce *et al.*, 2021).

Indeed, virtual morphometric approaches have the advantage of accounting for the whole bone shape and size variations, overcoming the possible limitations that may rise when focusing on single measurements only (e.g., Sorrentino *et al.*, 2020a). Prior studies involving three-dimensional statistical shape models (SSM) of fibulae (within the tibio-fibular complex), considering both fibular extremities and diaphysis, have found that sex contributes only subtly to the differentiation of fibular morphologies (Bruce *et al.*, 2021). Similarly, Tümer and colleagues (2019) did not find a consistent pattern of shape variation in relation to sex for the fibula. Furthermore, three-dimensional geometric morphometric (3D GM) studies of the whole fibula highlighted little to no morphological changes between sexes, mostly differing in fibular length but not significantly in terms of fibular shape, insufficiently to distinguish the sexes. Indeed, the authors found that fibulae were smaller in females than males. Interestingly, they also describe patterns of sex variations in relation to different ancestries (White, Black and Coloured individuals, the three main groups present in South Africa) that mostly relate to differences in

the contribution of body size to fibular shape among the different ethnicities of their subsamples, as Coloured individuals' smaller fibulae may possibly reflect the genetic influence of small-bodied Khoisan and Asian groups (Maass *et al.*, 2020). While differences in fibular shape also emerged among different ancestries, in their study, Maass and colleague (2020) adopted an approach based on only fixed landmarks, which accounted for the whole fibular length, with the consistent effect of possibly masking more subtle differences in epiphyseal shape and in general is highly influenced by the size component (Rohlf and Slice, 1990; Walker, 2000; Klingenberg, 2021).

In this work we apply for the first time a 3D GM approach that integrates fixed landmarks and sliding (semi)landmarks of curves and surfaces to investigate sexually dimorphic features in the fibular proximal and distal extremities. The study will involve three samples from identified modern skeletal collections (19th-20th cent.), two from Italian and one from South African populations. The main goal of this study is to provide a new method for sex assessment of the human fibular extremities based on 3D GM to be applied in forensic and bioarchaeological investigations. Based on previous studies on sexual dimorphism in the fibulae, we will test the two following hypotheses:

- (a) We expect to document variations of fibular epiphyseal form (i.e., size + shape) that may reflect a consistent functional differentiation of fibular extremities in relation to sex, with subtle sex-related differences in shape only (Maass *et al.*, 2020).
- (b) We hypothesize little to no variations in the shape the fibular epiphyses in relation to sex depending on different ancestries, but possible differences in form. We expect to find a similar degree of form sexual dimorphism among Italian and South African samples, but we also predict to highlight different patterns of epiphyseal size according to sex in relation to each population, due to a differentiation of body size among Italian and South African groups (Maass *et al.*, 2020).

2. Materials and methods

2.1. Sample

In this study we analyzed 136 left fibulae (Table 1) belonging to late 19th – early 20th century. The total sample consists of 17 South African individuals

from the Raymond A. Dart Collection of Human Skeletons at the University of the Witwatersrand (Johannesburg, South Africa) (Dayal *et al.*, 2009), and 119 Italian individuals (Emilia Romagna, N = 47; Sardinia, N = 72) (Italy) belonging to individuals from the Modern Human Identified Skeletal Collection of the University of Bologna. For the former sample, age at death for the whole collection spans 1920s to 2000s, while the latter samples, include individuals who died between 1898 and 1944 (Belcastro *et al.*, 2017). For each subsample (from now on population) the sex of the individuals is known from hospital or cemetery records, as well as other archival sources (e.g., birth certificates). Age-at-death spans between 17-90 years.

The fibulae used in this study were selected for their general good state of preservation and for the absence of pathological markers, with fully fused secondary centers of ossifications (Belcastro *et al.*, 2019).

The specimens were either digitized through computed tomography (CT) and laser scanning, as previous studies have shown that digital models of long bones from these two acquisition methods are comparable (Pietrobelli *et al.*, in press; Soodman *et al.*, 2018, Stephen *et al.*, 2020). The acquisition of the fibulae belonging to the Raymond A. Dart Collection was performed at the Microfocus X-Ray Computed Tomography facility of the University of Witwatersrand (Johannesburg, South Africa) on a Nikon Metrology XTH 225/320 LC (Voltage 70 kV, current 120 μ A, no filter used, pixel size 120 μ m). The acquisition of the fibulae of the Italian collections was carried out at Istituto Ortopedico Rizzoli (Bologna, Italy) utilizing a Revolution Discovery CT dual energy, with GSI Revolution and HD Revolution configurations (Voltage 100 kV, current 360 μ A, standard filter, slice thickness and acquisition interval at 0.625 mm, followed by a reconstruction with monochromatic beams at 40 keV, using the “Detail” filter).

A subsample of 20 Italian individuals of Bologna were digitized with ARTEC Space Spider 3D (Luxembourg) laser scanner (3D precision: 0.05 mm; 3D resolution: 0.1 mm) in the Department of Cultural Heritage of the University of Bologna. The generated surface model was exported in STL format (Artec Studio, 2013).

Data from CT scans were processed in Avizo 9.2 (Thermo Fisher Scientific,

Waltham) for image segmentation utilizing the threshold-based segmentation protocol of half-maximum height (HMH) outlined by Spoor, Zonneveld and Macho (1993). We applied the protocol following the modified version detailed in Coleman and Colbert (2007) and already used in several applications in anthropology (e.g., Pietrobelli *et al.*, in press; Zanolli *et al.*, 2018; Neil *et al.*, 2022). Then, a surface model was generated (isosurface reconstructions), saved in STL format, and loaded into Viewbox 4 (dHAL Software, Kifissia) for landmarking.

2.2. 3D Geometric Morphometric analysis

A 3D template configuration (Figure 1, Table 2) of 16 fixed landmarks, 25 curve semi-landmarks and 101 surface (semi)landmarks captured both the distal and proximal extremity of the fibula, as a modified version from the template used by Marchi *et al.*, (2022). The template was created in Viewbox 4, to cover major muscle, ligament and tendons attachment sites and articular surfaces on the fibula (Figure 1, Table 2). The Viewbox 4 software was utilized to apply the configuration to the targets in the sample, with (semi)landmarks sliding on curves and surface to minimize thin-plate spline (TPS) bending energy between the targets and the template (Slice, 2006). Following that, (semi)landmarks can be considered geometrically homologous among specimens (Gunz, Mitteroecker & Bookstein, 2005; Gunz & Mitteroecker, 2013). The template repeatability (i.e., high intra-observer agreement) and reproducibility with different scanning devices were tested in Pietrobelli *et al.*, 2021 (under review). Landmarks and (semi)landmarks raw coordinates used to describe the specimens of the study are available in Appendix S1 and S2. After importation of raw coordinates in R (version 4.0.3) (R Core Team, 2020), the set of (semi)landmarks of proximal and distal epiphysis were separate in two different datasets (i.e., a dataset of Cartesian coordinates for the distal and a dataset for the proximal fibular extremity). Each dataset was subjected to a further sliding step against recursive updates of the Procrustes consensus, while two different Procrustes superimpositions was computed, allowing the conversion of raw coordinates into standardized, scaled, centered and oriented shape coordinates (i.e., Procrustes coordinates) via Generalized Procrustes Analysis (GPA) (Slice, 2006; Rohlf & Slice, 1990) using the R package

“geomorph” version 3.3.2 (Adams *et al.*, 2018). Centroid size (CS) for each extremity, which is the square root of the summed squared distances between each (semi)landmark and the centroid of the (semi)landmark configuration was also calculated and used as a proxy of the size of the distal and proximal extremities (Slice, 2006).

Procrustes coordinates were then subjected to two separate Principal Component analyses (PCA) to explore shape variations based on different sex groups for both distal and proximal extremities separately, both considering each population separately and the pooled sample (Schlager, 2017). Two form-space (i.e., shape plus size) PCAs were computed by augmenting the Procrustes shape coordinates of each dataset of Procrustes coordinates by the natural logarithm of CS (lnCS) (Klingenberg, 2016). Visualization of extreme shape and form changes along the principal axes and means based on sex and populations groups were obtained by TPS deformation (Bookstein, 1991) of the Procrustes grand mean shape surface utilizing the R package “Morpho” v. 2.8 (Schlager, 2017). Linear correlation among CS and the scores along first two shape principal components of proximal and distal epiphyses was assessed by a Pearson’s correlation test, to assess whether the distribution of individual in the shape space PCA plot is influenced by size.

Procrustes ANOVA was adopted to test shape differences among sexes, both considering each population and the pooled sample, utilizing Procrustes distances among specimens and using a residual randomization procedure (RRPP = T, iterations = 1,000), with the R package “geomorph” version 3.0.7 (Adams *et al.*, 2018). Differences in size among sexes and populations were evaluated considering CS through ANOVA and subsequent post-hoc tests and visualized in relative box plots.

Allometric trajectories were estimated for both fibular epiphyseal extremities following Sorrentino and co-workers (2020a), by computing a multivariate regression of shape and form variables (using all the PCs) on CS. Then, all the obtained coefficients (slope and intercept) were utilized to compute a permutation test (N = 1000) on lengths (i.e., magnitude of the variability) and angles to assess significant differences (i.e., $p < 0.05$) among trajectories by populations and sexes group trajectories (Bailey, Benazzi & Hublin, 2014).

Finally, linear discriminant analysis (LDA) with leave-one-out cross validation testing assessed classification accuracy of sex based on shape variations using shape and form PC scores and centroid size to classify the specimens (i.e., either male or female). Selection within the first 10 PCs to include into LDA was based on an optimization between the amount of explained variance, considering 70% as threshold (Jolliffe, 2002) and the highest accuracy values obtained.

3. Results

3.1. Pooled sample

Italian populations (i.e., Emilia-Romagna, ER; and Sardinia, SAR) mostly overlap in shape space with each other and with South African individuals (SA) for both proximal and distal epiphyses. Additionally, no clear separation is present among individuals according to sex groups for any populations (Figure 2). For the proximal epiphysis, the first two principal components (PCs) accounts for 33.61% of total variance (PC1: 20.15%; PC2: 13.46%), while for the distal epiphysis the variance explained by the first two PCs is 56.41% (PC1: 33.76%; PC2: 22.65%). For both extremities, the first two PCs do not show a distinction among males and females ($p > 0.05$) with comparable males and females extreme shapes (Figure 2). Little to no morphological changes are seen in the extreme shape along PC1 and PC2 axes for both extremities (Figure S1) and mostly relates: for the proximal epiphysis, to a more pronounced styloid process towards positive scores and a more laterally protruding insertion of the m. fibularis longus towards negative scores of PC1 and PC2; for the distal epiphysis, to a more elongated and less pronounced area of tibio-fibular syndesmosis towards positive PC1 scores and negative PC2 scores, and to a more cranio-caudally elongated subcutaneous triangular surface along negative scores on PC1 and PC2. For the proximal epiphysis, no significant correlation is present between the first two PCs scores and CS. On the contrary, for the distal epiphysis a Pearson's correlation test shows that PC2 is significantly correlated with CS ($r = -0.565$; $p < 0.001$), indicating that static allometry could account for morphological differences in shape along this axis. Angles of allometric trajectories between populations groups do not differ significantly (Table 3) (Figure 2).

The form-space PCA plots of both extremities (Figure 3) show a clear separation among sexes, with males plotting towards positive scores along PC1 when considering all population pooled and grouped by sex. The first two PCs accounts for 49.05% of total variance for proximal epiphysis (PC1: 36.86%; PC2: 12.72%), while for distal epiphysis the first two components account for 73.34% of total variance (PC1: 58.35%; PC2: 14.99%). For both extremities, PC1 significantly contributes to the separation among males and females (proximal epiphysis: ANOVA; PC1: F-value = 88.39, $p < 0.001$; distal epiphysis: ANOVA; PC1: F-value = 18.26, $p < 0.001$), accounting mainly for size variation (i.e., CS). No distinction is present between sexes along PC2 or PC3 ($p > 0.05$). Generally, fibular epiphyses are smaller in females than in males, with narrower articular surfaces (Figure S2). For proximal epiphysis, males (PC1 positive) have a more pronounced styloid process, and a bulkier, laterally protruding fibular head, site of insertion of knee collateral ligaments and m. biceps femoris and fibularis longus, respectively, as compared to females. For distal epiphysis, males (PC1 positive) show a longer insertion of tibiofibular interosseous ligament, site of the tibiofibular-syndesmosis, longer subcutaneous triangular surfaces, peroneal grooves, protruding malleoli, attachments for calcaneofibular ligament and anterior and posterior talofibular ligaments, and a deeper and wider malleolar fossa, attachment for the transverse tibiofibular and posterior talofibular ligaments in comparison to females. Angles of allometric trajectories between groups do not differ significantly according to different populations (Table 3).

For both extremities, CS is significantly different between pooled males and females (proximal epiphysis: ANOVA; F-value = 89.39, $P < 0.001$; distal epiphysis: ANOVA; F-value = 7.38, $P < 0.001$) (Figure 4).

Cross-validation LDA of the pooled sample reaches an accuracy above 80% when using the first 6 form space PCs (76.87% of variance explained) and the first 4 form space PCs (83.38% of variance explained) for proximal and distal epiphyses, respectively (Table 4). Using CS, accuracy is between 68-76%, considering both epiphyseal ends. Fewer individuals are correctly classified according to sex when using shape space PCs, with an accuracy of 57-62% using 10 PCs (82.05% and 85.12% of variance explained for proximal and distal

epiphysis respectively) (Table 4).

3.2. Separate populations

Figure 5 and Figure 6 show the shape space PCA plots of proximal and distal fibular extremities, respectively, for each population grouped by sex and their related mean shape. In all populations and for both fibular extremities, PCs scores are not significantly correlated ($-0.5 < r < 0.5$) with CS, except for PC2 in SA for distal epiphysis ($r = -0.706$, $p = 0.002$).

In general, only subtle shape morphological changes are seen among mean shapes of males and females of each population and are mostly related to a slightly wider antero-posterior expansion, with longer malleoli in males in comparison with females, which possess, for ER, a shallower malleolar fossa for distal epiphyses. In shape-space, indeed, PC scores along the first three PC axes do not contribute to the separation among males and females in any of the three populations (ANOVA, $p > 0.05$) for both proximal and distal epiphyses, except for the distal extremity of ER which show weak significant difference between males and females (ANOVA, PC1: F-value = 4.21, $p = 0.046$). Angles of shape allometric trajectories do not differ significantly according to sex considering each population separately (Table 5).

Figure 7 and Figure 8 show the scatterplot of form-space PCA plot of proximal and distal fibular extremities, respectively, for each population grouped by sex and their related mean shape. For all populations, PC1 shows the main separation between sexes as accounting for size variation (i.e., CS represented by PC1).

For the two Italian populations males plot towards PC1 positive scores and females plots towards negative scores (Figures 7 and 8), although slightly overlapping especially on distal fibula extremity (Figure 8). ANOVAs show significant separation ($p < 0.05$) along PC1 between sexes for both proximal and distal epiphyses for the two Italian populations but not for South Africans. For distal epiphyses, moreover, Italian ER population also presents a significant separation according to sex along PC2 (ER: PC2, F-value = 15.54, $p < 0.001$).

For proximal epiphysis (Figure 7), Italian males show wider fibular heads with more antero-posteriorly expanded tibio-fibular articular surfaces and a more pronounced styloid process than females. In addition, males possess an area

of insertion of knee collateral ligaments, m. biceps femoris and fibularis longus that is more postero-laterally protruding than in females, especially evident in SAR population. For distal epiphysis (Figure 8), Italian males exhibit more medio-laterally expanded proportions, with longer and pronounced malleoli, attachment for calcaneofibular ligament and anterior and posterior talofibular ligaments, and a deeper and wider malleolar fossa, attachment for the transverse tibiofibular and posterior talofibular ligaments, ankle strengtheners, in comparison to females. On the contrary, South African males do not show such marked pattern of form variation, with comparable mean form morphologies seen in both sexes. Angles of form allometric trajectories do not differ significantly according to sex considering each population separately (Table 5).

For both extremities, CS is significantly different between males and females of the two Italian populations (ANOVA, ER and SAR: $p < 0.05$) but does not significantly discriminate between sex groups in South Africans (Figure 9). For proximal epiphyses, females of all populations do not differ among one another, and so do SA and SAR males, who differ from ER but not between each other. For distal epiphysis, ER females and males differ from their SAR counterparts, while both Italian males and females do not differ significantly with their SA equals.

Table 6 shows cross-validation LDA accuracy percentages of sex determination by populations for both fibular epiphyses. Shape PCs provide less discriminatory power in all three populations, with accuracy percentages of correct sex estimation using proximal epiphysis spanning 40-54% in Italians and 60% in South Africans. Similarly, for distal epiphyses accuracy is lower for shape PCs, ranging 51-72%. As regards form PCs and considering both extremities, in ER the first 3-5 PCs (76.58%-77% of variance explained) provide 89-93% accuracy in sex determination; in SAR the first 4-6 PCs (84.76%-77-71% of variance explained) provide 80-83% accuracy in sex determination. Accuracy percentages of LDA on form PCs on South Africans are sensibly lower (including both epiphyses, 50-53%, with 77.4%-79.2% of variance explained considering the first 4 PCs). Centroid size separate sexes — considering both epiphyses — with 69-87% accuracy for Italian populations, and with 66-68% accuracy for

South African populations.

4. Discussion

The aim of this study was to highlight patterns of sexually dimorphic form variations in the fibular proximal and distal epiphyses in individuals of three different populations from Italy and South Africa belonging to identified modern skeletal collections (19th-20th century) utilizing a 3D GM approach. Our study provides a new method for sex estimation on the fibula, which remains scarcely analysed in anthropology (e.g., Marchi *et al.*, 2022), despite its potential persistence in bioarchaeological (Willey *et al.*, 1997) and forensic (Crowder & Austin, 2005) contexts.

We hypothesized the presence of variations of fibular epiphyseal size in relation to sex. Results from our analyses supports a distinction among sexes, mainly more robust epiphyses in males than in females and especially in the Italian samples. Our second hypothesis was that scarce sex variations would have been present in the shape of the fibular epiphyses in relation to sex depending on different ancestries and mostly refer to different patterns of epiphyseal size in different populations, due to a differentiation of body size among Italian and South African groups. Our results support the former point and provide only partial support to the latter one. In fact, fibular extremities form and size significantly distinguish between sexes in the Emilia Romagna (ER) and Sardinia (SAR) populations but not in the South African population. Overall, our results show that fibular extremities account for subtle shape changes, while significant form and size differences are present between sexes, even with differences related to ancestry. The sex determination method provided in this paper, based on cross-validation LDA on form PCs, provides an accuracy above 80 % when the samples are pooled and reaches an accuracy of 80-93% when Italian populations are considered separately. However, the method was not successful for the South African sample (50-53% accuracy).

Our results are consistent with previous investigation on sex determination using the fibula, adopting both traditional metrics (Sacragi & Ikeda, 1995; Naidoo *et al.* 2015; Krüger *et al.* 2017; Fasemore *et al.*, 2018) and virtual methods (Tümer *et al.*, 2019; Nolte *et al.*, 2020; Audendart *et al.*, 2019; Bruce

et al., 2021; Maass *et al.*, 2020). Specifically, we obtained similar results to previous studies that utilized 3D GM methods on the whole fibula (Maass *et al.*, 2020), which highlighted different degrees of size dimorphism between males and females, but no significant shape differences between the sexes were observed once size information is removed.

In our study, morphological differences of both extremities in form space suggests that females possess smaller articular surfaces, shorter and narrower malleoli, shallower malleolar fossa, with subsequently reduced area of insertion of knee and ankle collateral ligaments, and less robust muscle insertions for the m. fibularis longus, m. biceps femoris and interosseous ligament distal attachment (Figure 3; Figure S2; Figure 7-8). This indeed may reflect a sex-specific pattern of knee (Dargel *et al.*, 2011; El Ashker *et al.*, 2017, Li *et al.*, 2018; Yurek *et al.*, 2021) and ankle/foot morphology and posture (O'Connor *et al.*, 2006; Sorrentino *et al.*, 2020a; Harris & Troy Case, 2012), and in general support the existence of sex-segregated patterns of lower limb muscle activation in different sexes (Edwards & Jakobi 2006; 2007; Harwood *et al.*, 2007; Harwood, Edwards, Jakobi, 2008). Regarding the ankle, previous studies have demonstrated sex-related differences in anatomical and biomechanical features of the joint (Yurek *et al.*, 2021). Adjei, Nalam & Lee (2020), coherently with Trevino and Lee (2018), found that ankle stiffness varied significantly between sexes, both along the sagittal and frontal planes (therefore concurring in dorsiflexion/plantarflexion and inversion/eversion, respectively), with greater joint stiffness in females both while quiet standing and muscle activation occurred. The authors explains their findings with the greater passive resistance of females to a greater range of motion, lower elastic modulus, and an higher ligamentous laxity (Wilkerson & Mason, 2000; Beynnon *et al.*, 2001; Kubo *et al.*, 2003) than males, which by contrast possess more leg muscle mass (Janssen *et al.*, 2000) and a higher muscle and cortical bone cross-sectional area in the whole lower limb (Miller *et al.*, 1993; Ruff & Hayes, 1983; Ruff, 1987) than females. Females have an increased range of motion in the sagittal plane of rearfoot and midfoot, peak plantarflexion angle of rearfoot, peak dorsiflexion and abduction angle of midfoot, as compared with males (Takabayashi *et al.*, 2017). Females also possess greater

ligamentous laxity, resulting subsequently in this increased mobility of the ankle joint (Beynon *et al.*, 2005; Wilkerson & Mason, 2000). Indeed, our results show that females show shallower malleolar fossae with reduced area of insertion of ankle stabilizers (transverse tibiofibular and posterior talofibular ligaments), less protruding malleoli with reduced area of insertion of ankle collateral ligaments (anterior, posterior talofibular and calcaneofibular ligaments) and less robust muscle insertions for the m. fibularis longus, the main evertor of the ankle (Figure 3; Figure S2; Figure 7-8). This result seems also to indicate sex-specific differences in structural properties of ankle stabilizers, as differences in tendons tissue properties of the leg already emerged (Kubo *et al.*, 2003).

Regarding the knee, important differences among sexes have been established in morphology and kinematics (Parker and Hagen, 2021). El Ashker and colleagues (2017) found that males have higher functional hamstring-to-quadriceps strength ratios than females, suggesting that possible quadriceps dominance in females or greater contribution of the hamstrings in males than in females either in motion or during foot contact with the ground, stabilising the joint angle. Their findings seem coherent with our result, showing a protruding area of attachment of m. biceps femoris in the proximal fibular extremity in males (Figure 3; Figure S2; Figure 7-8). While the specific relationship between a muscle and its tendon can change depending on several factors (e.g., muscle/tendon specificity, loading degree, age), in our sample such variations are minimal, since all individuals possess similar activity level (i.e., sedentary lifestyle, Sorrentino *et al.*, 2020b) and age variations in the samples are similar (Table 1). Therefore, tendon size could be used to assess muscle size (Marchi *et al.*, 2022). Finally, we did not find significant shape/form sex-related variation regarding articular orientation in agreement with previous studies on sex variations of the bones of the leg (Bruce *et al.*, 2021; but also see Degen *et al.*, 2020).

Our results show only weak sex-related differences in fibular muscle and ligaments insertions when size is excluded from analysis (Figure 2; Figure S1; Figure 5-6). It is interesting to notice that studies have found higher mean torque of all major muscle groups in the lower extremity in men (women < 62–

70% of men), but when measurements are standardized by size (i.e., bone mass index or body weight) no significant differences in strength, endurance, or torque between sexes have been found (Sepic *et al.*, 1986 *cercare alra biblio*). However, when body surfaces and dimensions are evaluated aside from muscle attachments, the relationship between sex, shape and size is more complex: Wunderlich and Cavanagh (2001) stressed that female lower limbs are not solely scaled down versions of the male lower limb. Particularly, the lateral side of the foot, directly involved in eversion, showed marked sex related differences even after standardization of size (O'Connor *et al.*, 2006). Indeed, our analysis indicates consistent differences between males and females in size, as sexes differ according to CS in the two Italian populations and when the sample is pooled. According to our results, fibulae of males possess larger linear dimensions than females, as a results of a generally larger body size, possibly due to a relatively longer pubertal growth period, and the subsequent development of larger bones and muscles under the influence of sex genes, sex steroids (androgens and estrogens) and other hormones, such as growth hormone (GF) and insulin-like growth factor 1 (IGF1) which, concurring with mechanical loading, may further contribute to the development of the skeletal sexual dimorphism (Callewaert *et al.*, 2010 Scheuer & Black 2000; López-Costas *et al.*, 2012). Indeed, bone growth is differentiated between sex groups, mostly evident during puberty but to a lesser extent also apparent as early as early childhood (Malina & Johnston, 1967; Humphrey, 1998; Stull *et al.*, 2017) and concur to the observed greater cortical bone plasticity in males, modelled by greater muscle mass during ontogeny, determining greater long bones lengths and breadths, also already observed for the fibula in a similar sample (Pietrobelli, Marchi & Belcastro, 2022). During puberty, males reach higher peak bone mass, greater bone size and, ultimately, a stronger skeleton than females (Seeman, 2001) with different skeletal maturation timing for both fibular extremities according to sex (proximal epiphyses: 12-17 years for females and 15-20 years for males (McKern & Stewart, 1957); distal epiphyses: 12-16 years for females and 14-19 years for males (Crowder & Austin, 2005)). Our results are also coherent with already observed different sizes in males and females for the whole size of

tibio-fibular complex (Audenaert *et al.* 2019; Bruce *et al.*, 2021) and with prior assessments on the fibula alone (Maass *et al.*, 2020).

The present study highlights the importance of a population-based approach for sexing the human fibula, as our result point to a different degree of sexual dimorphism in populations with different ancestries (Figure 7-8; Figure 9). Maass and colleagues (2021) found that Coloured males and females differed significantly from Black and White males and females, with the latter groups not differing from one another, and interpreted their results in light of the genetic contribution of small-bodied groups within the Coloured group. However, in their case, males and females had significantly different size within the three populations, considered separately. Our results comparing different ancestries, on the other hand, show that sexual variations in fibular extremities are not strictly related to size: in fact, while Sardinians and South Africans have similar (smaller) fibular epiphyseal sizes, the former show a difference in size among sexes, while the latter do not (Figure 9). Some fluctuations in the degree of sexual dimorphism among different population is expected and could be ascribed to either proximity among individuals of the same population (i.e., response to nutritional stress or overall improvements in the environment) and ultimate causations (i.e., selection and genetic adaptation to a variety of ecological, social, or economic factors) (Freyer & Wolpoff, 1985; Lazenby, 2002).

Fibular extremities appear more dimorphic in the Italian samples than in the African one, likely as the results of the small sample size characterizing the South African sample or for other reason which are not explored in this study. This result needs to be further investigated by increasing the sample size and including populations with different ancestry, subsistence strategies and lifestyles that may led to differences in the shape of the bones (Sorrentino *et al.*, 2020b). However, the 89-93% accuracy in sex determination observed for the Italian samples shows the potentiality of the method presented in this study and is coherent with similar discriminant functions provided by studies on fibular sexual dimorphism (Sacragi *et al.*, 1993; Spradley & Jantz 2011; Spradley *et al.*, 2015; Moore *et al.*, 2016; Krüger *et al.*, 2017; Sacragi & Ikeda 1995; Naidoo *et al.*, 2015; Mazenganya & Billings 2016; Fasemore *et al.*, 2018),

mostly reaching the threshold of 80% suggested on sex estimation using long bones (Krogman & Iscan, 1986). Indeed, when form PCs do not differ significantly among sexes, accuracy percentages are much lower. When size is considered aside from shape variations (CS), accuracy percentages are quite reduced (69-87%), suggesting that the interaction between size and shape (i.e., form) mainly account for sex difference in the human fibula.

The main limitation of this study is the low numerosity of the South African sample, which may have influenced the lack of significance in all comparisons performed in our study. A further confounding factor is the South African sample may be the mixed origins of the populations included in this study (Dayal *et al.*, 2009). Moreover, other factors may have contributed the lack of sex variation in fibula, such as genetic background, environmental changes and changes in socio-economic and health conditions, as already observed in the larger bones of lower limb (Prince, Cameron and Tobias, 1987). Even though previous GM results on the fibula indicate a lack of sexual dimorphism in the fibula (Maass *et al.*, 2020), the different methodology here employed (with sliding semi-landmarks instead of only fixed landmarks) could better detect subtle differences. Further studies are therefore necessary to evaluate in more detail this aspect in the South African population.

5. Conclusion

In this study we provided a novel methodology that can be used to determine sex in both bioarchaeological and forensic investigations using the proximal and distal extremities of the human fibula, rarely analysed in anthropology. To our knowledge, this is the first study that address fibular epiphyseal shape, form and size in relation to sexual dimorphism, adopting a 3D GM method that includes both fixed landmarks and sliding (semi)landmarks, that together account for detailed morphological variations of fibular extremities. Our work included three different populations, two from Italy and one from South Africa belonging to identified modern skeletal collections (19th-20th century).

The results of the 3D GM study of the proximal and distal extremities of the fibula showed that the fibula can be used to assess sex with an accuracy ranging from 80% to 93% in the two Italian populations. The differences mainly consist in larger and more robust epiphyses in males than in females, with

females showing narrower articular surfaces, shorter and narrower malleoli, shallower malleolar fossa and peroneal groove, and less protruding muscle insertions for the m. fibularis longus, m. biceps femoris and interosseous ligament distal attachment. Such distinct form morphology may reflect a sex-specific pattern of knee and ankle/foot posture, with distinctive lower limb morpho- functional aspects in relation to sex. Our results also highlight the importance of a population-based approach to sex determination of the human fibula, as evidenced by our finding that sexual dimorphism can be determined for the two Italian populations but not for the South African one. This may suggest a different degree of sexual dimorphism in populations with different ancestries, that is not strictly related to size variations. However, when the sample is pooled, our sex estimation method based on cross-validation LDA on form PCs gives an accuracy above 80%, which is lower when considering the size alone and the shape PCs, suggesting that the interaction of size and shape is the pattern that mainly reflect sexual dimorphism in the human fibula.

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Data availability statements

Raw landmarks coordinates and sample list of individuals included in the present study are available as Supplementary Materials. All R scripts utilized for the analyses are available upon request to the corresponding authors.

Conflict of interest

The authors declare no potential conflict of interest.

Authors contributions

AP conceptualization, investigation, formal analysis, validation, writing-original draft, methodology, writing-review, and editing; RS validation, writing-original draft, methodology, writing-review, and editing; SD methodology, data acquisition, validation, software; DM, SB, MGB conceptualization, supervision of the research, methodology, project administration, software, writing-review and editing.

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Tables

Population	Age Range	Females	Males	Total
Emilia-Romagna (MHISC UBOL *)	18–84	24 (18%)	23 (17%)	47
Sardinia (MHISC UBOL)	17–88	29 (21%)	43 (31%)	72
South Africa (Dart Collection)	20–90	10 (7%)	7 (5%)	17
Total	17–90	63 (46%)	73 (54%)	136

Table 1. Sample composition by populations, age range, and sex. * Modern Human Identified Skeletal Collection of the University of Bologna.

Landmarks	Definition	
L1	Point where the <i>fibular anterolateral border</i> divides into two ridges: the proximal apex of the <i>subcutaneous triangular surface</i> (STS)	
L2	Most medial point of the medial border of the STS	
L3	Most lateral point of the lateral border of the STS	
L4	Most distal point of the <i>lateral malleolus</i> in anterior view	
L5	Most distal point of the posterior border of the <i>malleolar fossa</i>	
L7	Most anterior point on the anterior border of the <i>proximal fibular-talar articular facet</i> (PAF)	
L8	Point between the anterior border of PAF and the <i>anterior border of distal fibular-talar articular facet</i> (DAF)	
L9	Most distal point of DAF	
L10	Most proximal point on the posterior border of DAF	
L12	Most posterior point of the proximal border of PAF	
L13	Most proximal point of <i>proximal tibiofibular articular facet</i>	
L14	Most proximal point of <i>interosseous tibiofibular ligament</i> (ILA) insertion	
L15	Most proximal point on styloid process of fibular head in medial view	
L16	Most anteroproximal point on anterior border in medial view (above fibular neck)	
L17	Most posteroproximal point on posteromedial border in medial view (above fibular neck)	
L18	Most posteroproximal point on posterior border in lateral view (above fibular neck)	
Curves	Definition	N
C_1->2	Medial border of the <i>subcutaneous triangular surface</i> (STS)	5
C_1->3	Posterior border of the STS	7
C_7->8	Anterior border of the <i>proximal fibular-talar articular facet</i> (PAF)	1

C_8->9	Anterior border of the <i>distal fibular-talar articular facet</i> (DAF)	1
C_9->10	Posterior border of the DAF	1
C_10->12	Posterior border of the PAF	1
C_8->10	Border between the PAF and DAF	1
C_13->13	Outline of <i>proximal tibiofibular articular facet</i>	6
C_7->12	Proximal border of the PAF	2
Surfaces	Definition	N
SSML_malleolar fossa	Surface of the <i>malleolar fossa</i> , attachment site of the <i>transverse tibiofibular</i> and <i>posterior talofibular ligaments</i> .	7
SSML_ILA	Attachment surface of <i>interosseous tibiofibular ligament</i> and part of <i>interosseous membrane</i> (ILA)	13
SSML_fibular groove	Groove for tendons of <i>m. peroneus longus</i> and <i>m. tertius</i> and attachment site of <i>posterior tibiofibular ligament</i>	13
SSML_STS	<i>Subcutaneous triangular surface</i> (STS)	24
SSML_FiTal1Ar	Proximal <i>fibular-talar articular facet</i> (PAF)	4
SSML_FiTal2Ar	Distal <i>fibular-talar articular facet</i> (DAF)	3
SSML_head	Proximal <i>tibiofibular articular surface</i>	5
SSML_prox_ep	Surface of <i>proximal epiphysis</i>	32

Table 2. Fibular landmarks and (semi)landmarks identification, definition, and number.

Pooled Sample			
		Allometric Trajectories	
		Shape	
		α (°)	p
Proximal	ER-SAR	95.95	0.190
	ER-SA	54.24	0.987
	SAR-SA	84.93	0.548
Distal	ER-SAR	29.75	0.381
	ER-SA	35.99	0.792
	SAR-SA	35.84	0.520
		Form	
		α (°)	p
Proximal	ER-SAR	16.92	0.234
	ER-SA	24.38	0.305
	SAR-SA	25.54	0.440
Distal	ER-SAR	9.47	0.426
	ER-SA	11.96	0.799
	SAR-SA	12.05	0.580

Table 3. Allometric trajectory comparisons among different populations computed with the pooled sample for proximal and distal fibular epiphyses in shape and form space. α (°) indicates the angle of divergence of each trajectory in pairwise comparisons, with relative p-value (p).

Pooled Sample			
<i>Shape</i>			
	PCs	N	%acc
<i>Proximal</i>	10	76/133	57
<i>Distal</i>	10	85/163	62
<i>Form</i>			
	PCs	N	%acc
<i>Proximal</i>	6	107/133	80
<i>Distal</i>	4	111/136	81
<i>Csize</i>			
	N		%acc
<i>Proximal</i>	102/133		76
<i>Distal</i>	93/136		68

Table 4. Sex determination accuracy percentages obtained by cross-validation LDA on the pooled sample (Emilia-Romagna, Sardinia, and South Africa) for shape and form PC scores (PCs) and centroid size (CS) of proximal and distal epiphyses.

Separate Populations				
Allometric Trajectories M-F				
<i>Shape</i>				
	<i>Proximal</i>		<i>Distal</i>	
	α (°)	p	α (°)	p
ER M-F	76.44	0.543	31.39	0.548
SAR M-F	54.38	0.978	36.31	0.347
SA M-F	122.44	0.05	89.34	0.242
<i>Form</i>				
	α (°)	p	α (°)	p
ER M-F	28.33	0.604	14.43	0.658
SAR M-F	16.17	0.99	13.92	0.381
SA M-F	140.21	0.195	43.16	0.215

Table 5. Allometric trajectory comparisons of males and females computed for each population separately, for proximal and distal fibular epiphyses in shape and form space. α (°)

indicates the angle of divergence of each trajectory in pairwise comparison, with relative p -value (p).

Emilia-Romagna				Sardinia			South Africa		
Shape									
	PCs	N	%acc	PCs	N	%acc	PCs	N	%acc
Proximal	10	19/47	40	7	39/71	54	4	9/15	60
Distal	5	24/47	51	7	52/72	72	5	9/16	56
Form									
	PCs	N	%acc	PCs	N	%acc	PCs	N	%acc
Proximal	5	42/47	89	6	49/71	80	4	8/15	53
Distal	3	44/47	93	4	60/72	83	4	8/16	50
CS									
		N	%acc		N	%acc		N	%acc
Proximal		41/47	87		49/71	69		10/15	66
Distal		34/47	72		50/72	69		11/16	68

Table 6. Sex determination accuracy percentages obtained by cross-validation LDA on each population for shape and form PC scores (PCs) and centroid size (CS) of proximal and distal epiphyses.

Figures

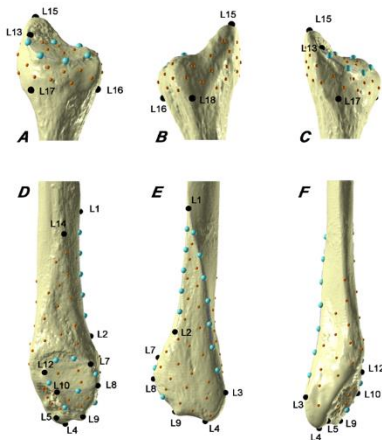


Figure 1. Landmarks (black), curve (light blue), and surface (semi)landmarks (orange) digitized on a left fibula (A), proximal fibula, medial view; (B), proximal fibula, lateral view; (C), proximal fibula, posteromedial view; (D), distal fibula, medial view; (E), distal fibula, lateral view; (F), distal fibula, posterior view. See Table 2 for a detailed description of the anatomical landmarks.

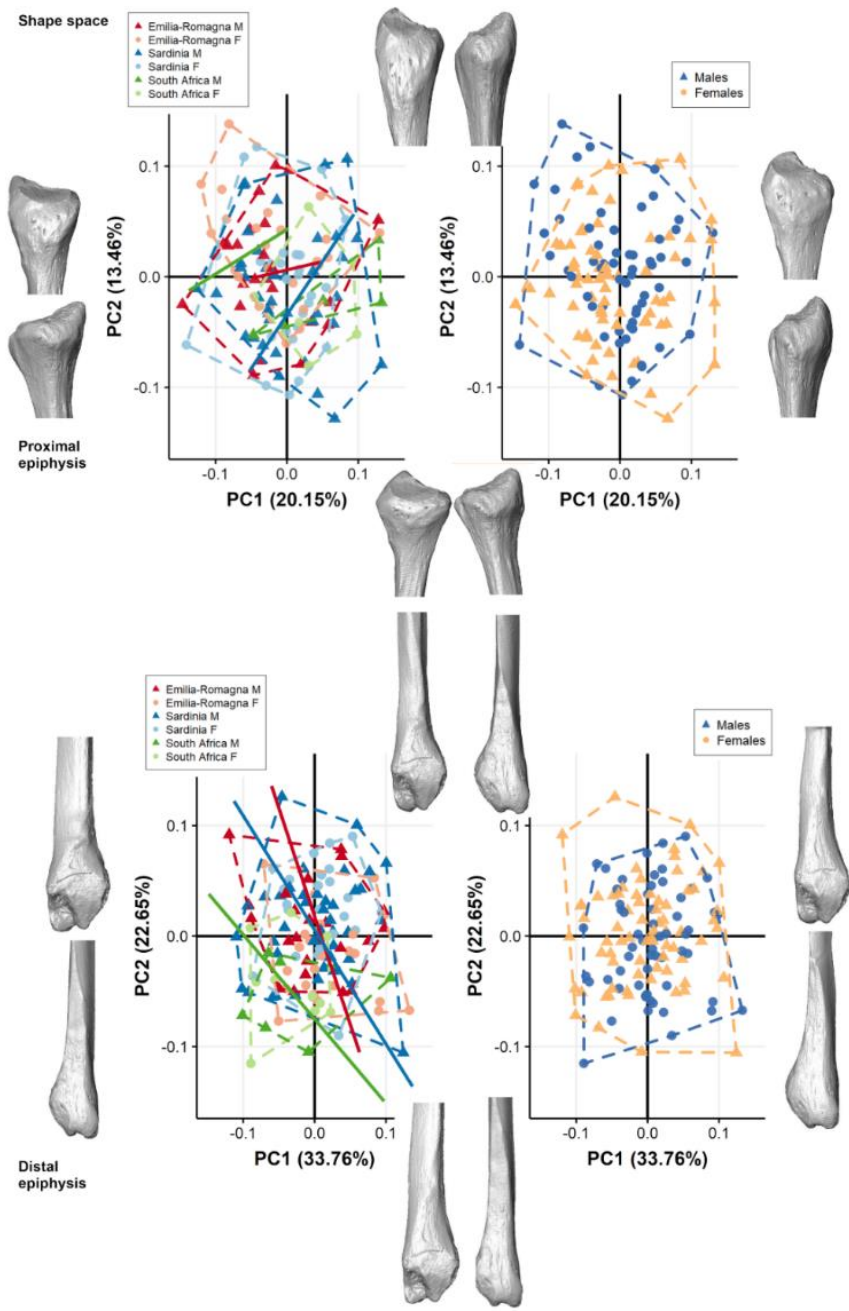


Figure 2. Scatterplot of shape-space PC scores for both proximal and distal epiphyses computed considering all populations pooled together, grouped by population (on the left) and by sex (on the right). For both proximal (above) and distal (below) extremities, positive and negative extreme shape variations along PC1 (right and left) and PC2 (top and bottom) are represented. Lines represent allometric trajectories for all populations. Allometric trajectories for ER (red line), SAR (blue line), and SA (green line) are represented.

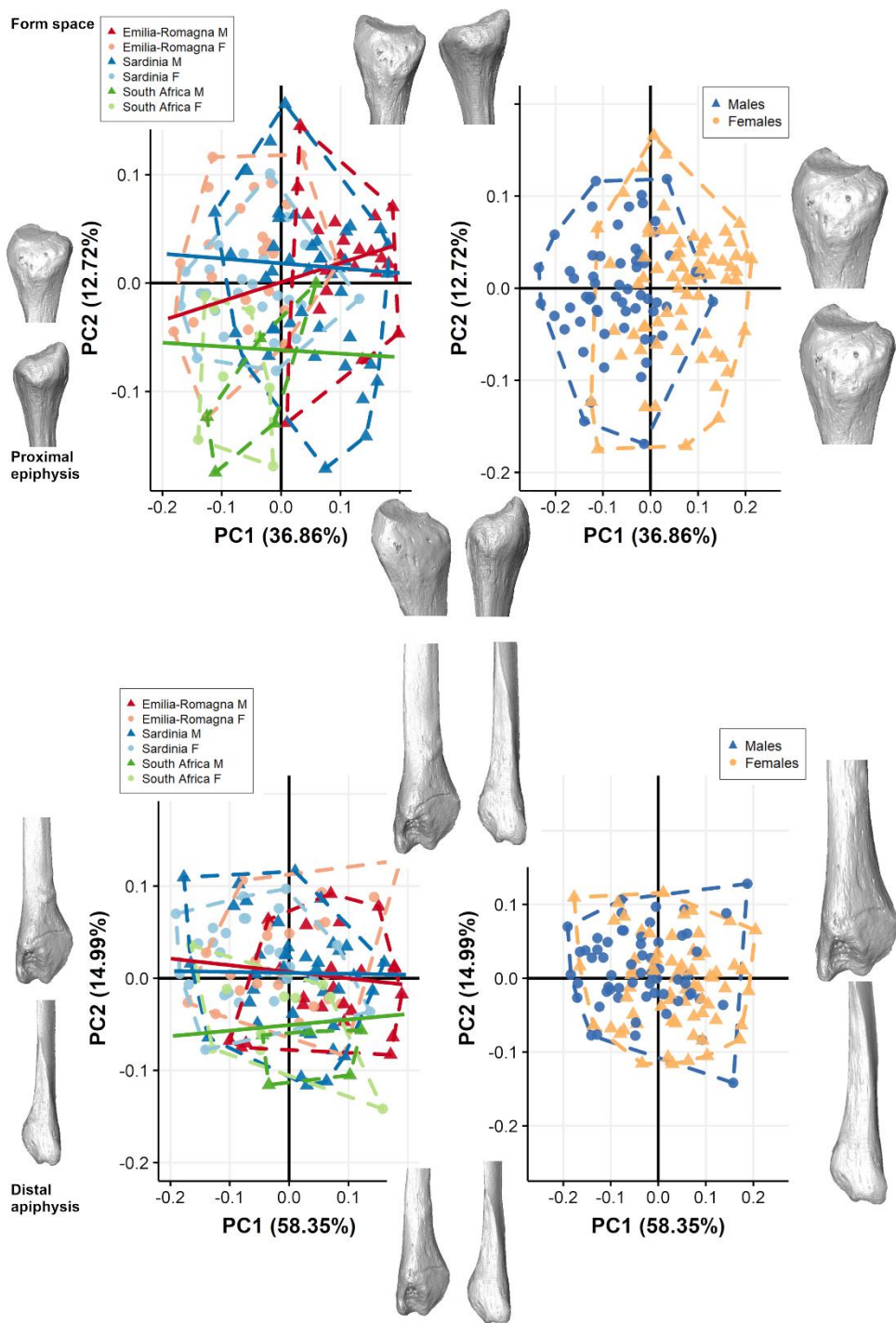


Figure 3. Scatterplot of form-space PC scores for both proximal and distal epiphyses computed considering all populations pooled together, grouped by population (on the left) and by sex (on the right). For both proximal and distal extremities, positive and negative extreme form variations along PC1 (right and left) and PC2 (top and bottom) are represented. Lines represent allometric trajectories for all populations. Allometric trajectories for ER (red), SAR (blue), and SA (green) are represented.

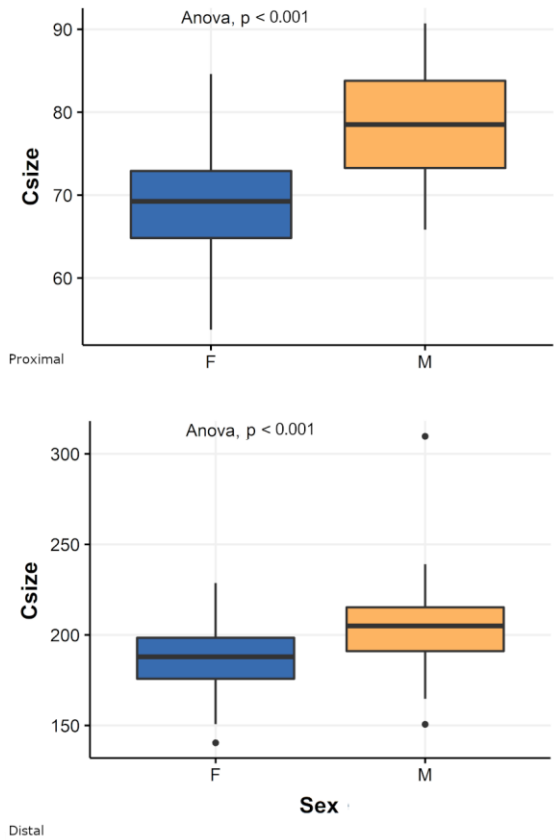


Figure 4. Box plot of centroid size (Csize) of proximal (above) and distal (below) fibular epiphyses for the pooled sample by sex. M = males; F = females. Black lines are the medians, boxes represent the interquartile ranges, whiskers the non-outliers range and filled circles the outliers.

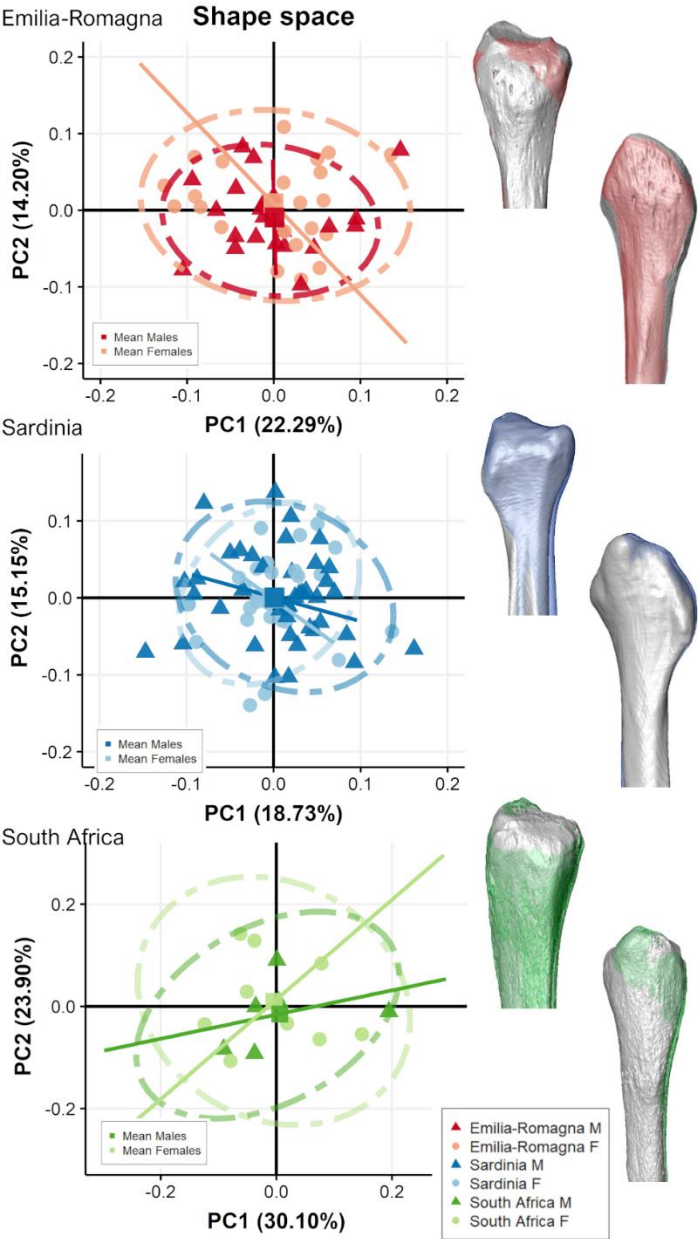


Figure 5. Scatterplot of shape-space PC scores of proximal fibular epiphyses for the two Italian (Emilia-Romagna and Sardinia) and the South African populations, divided by sex and presenting mean male (colored) and female (grey) shape variation along the PC axes, represented in the scatterplot as squared dots, in medial (top) and lateral (bottom) views. Lines represent allometric trajectories for males and females for each population.

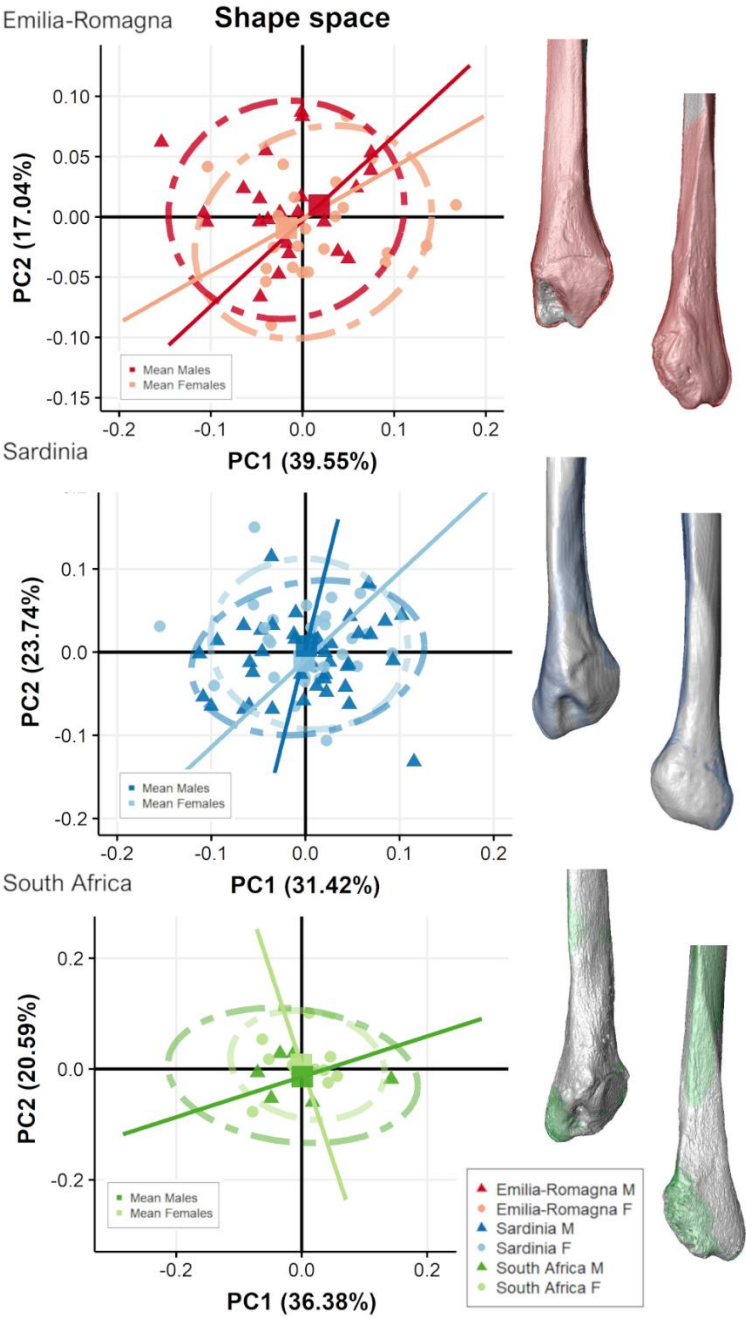


Figure 6. Scatterplot of shape-space PC scores of distal fibular epiphyses for the two Italian (Emilia- Romagna and Sardinia) and the South African populations, divided by sex groups and presenting mean male (colored) and female (grey) shape variation along the PC axes, represented in the scatterplot as squared dots, in medial (top) and lateral (bottom) views. Lines represent allometric trajectories for males and females for each population.

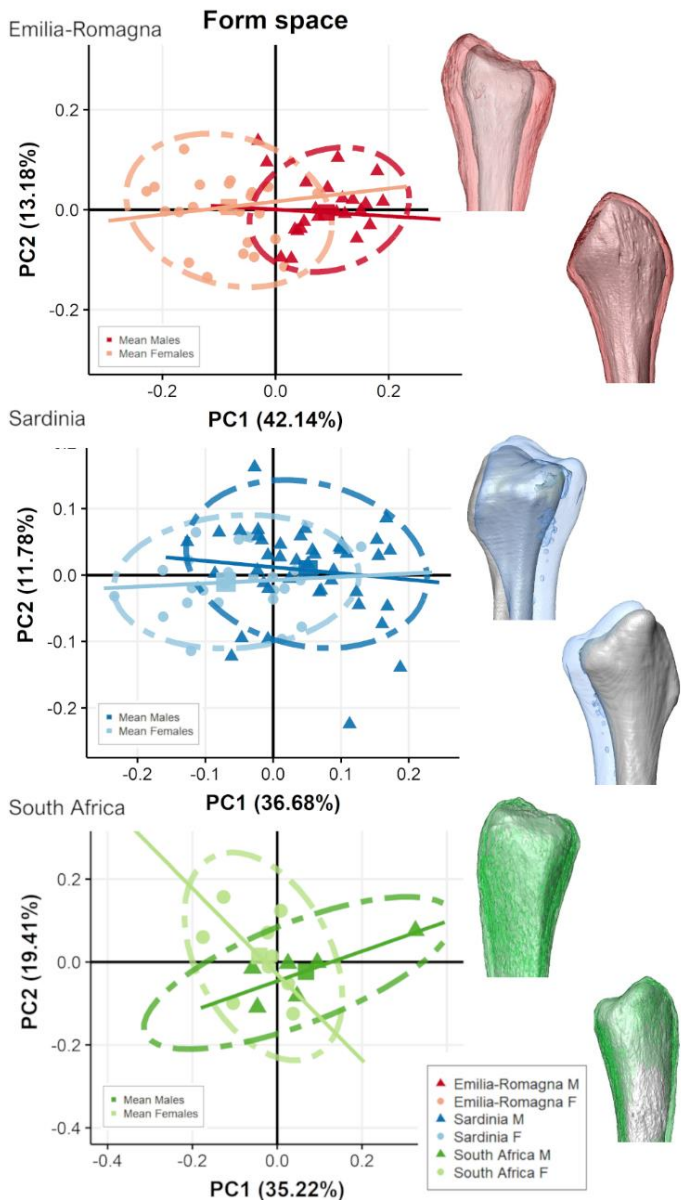


Figure 7. Scatterplot of form-space PC scores of proximal fibular epiphyses for the two Italian and the South African populations, divided by sex groups and presenting mean male (colored) and female (grey) form variation along the PC axes, represented in the scatterplot as squared dots, in medial (top) and lateral (bottom) views. Lines represent allometric trajectories for males and females for each population.

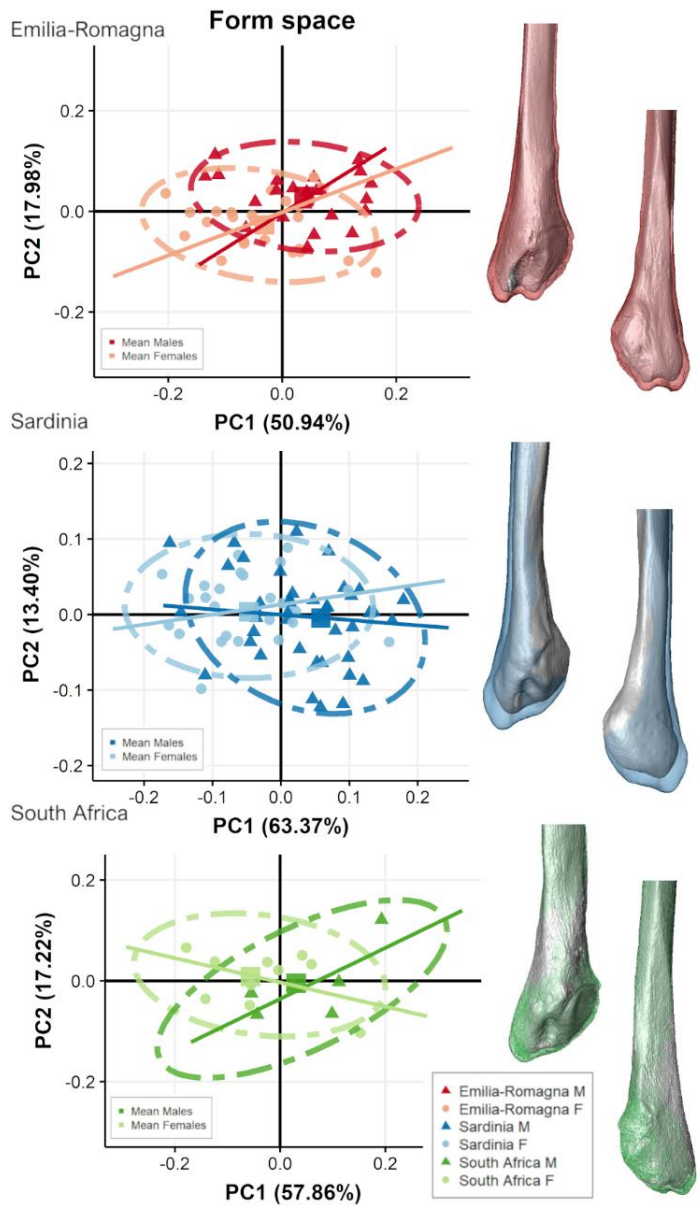


Figure 8. Scatterplot of form-space PC scores of distal fibular epiphyses for the two Italian and the South African populations, divided by sex groups and presenting mean male (colored) and female (grey) form variation along the PC axes, represented in the scatterplot as squared dots, in medial (top) and lateral (bottom) views. Lines represent allometric trajectories for males and females for each population.

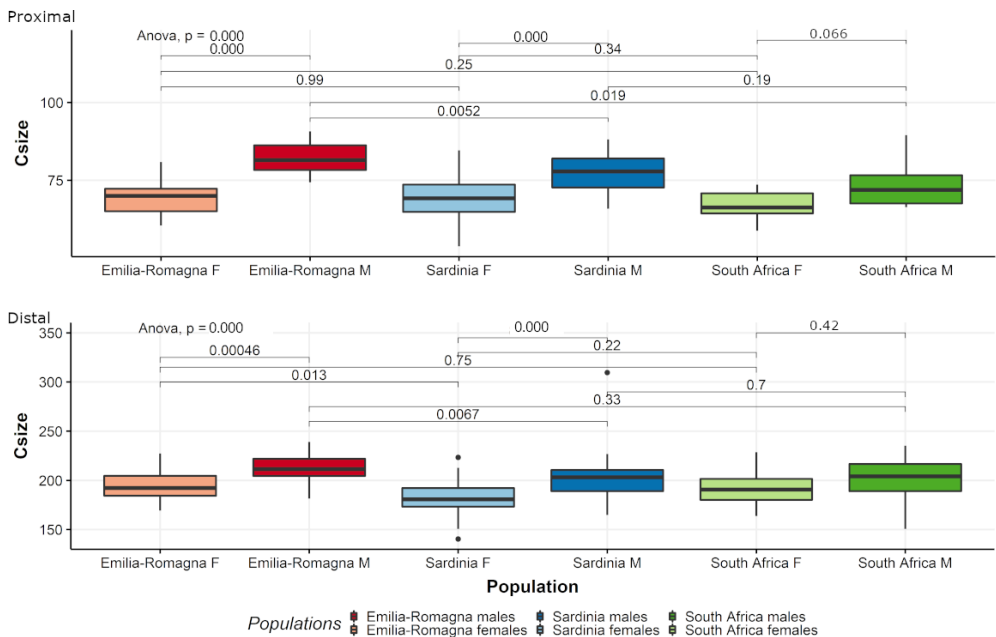


Figure 9. Box plot of centroid size (Csize) of proximal (above) and distal (below) fibular epiphyses all populations by sex. M = males; F = females. Black lines are the medians, boxes represent the interquartile ranges, whiskers the non-outliers range, and filled circles the outliers

Supplementary materials are available at:

<https://www.mdpi.com/article/10.3390/biology11071079/s1>



PAPER II

*Interpopulation
variation:
subsistence,
terrain, shoe use*

CONTENTS

Pietrobelli A, Sorrentino R, Sparacello V, Benazzi S, Marchi D, Belcastro MG (2023), Exploring the role of fibular extremities as indicators of mobility patterns and locomotor strategies in *Homo sapiens* from Mid-Upper Paleolithic to modern age. *In preparation*

Title:

Exploring the role of fibular extremities as indicators of mobility patterns and locomotor strategies in *Homo sapiens* from Mid-Upper Paleolithic to modern age.

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Abstract

The human fibula has been scarcely analyzed in biological anthropology. However, studies on athletes and human archaeological samples suggest the importance of including fibular structural properties to reconstruct mobility patterns, locomotor activities and strategies in past populations. This study analyzes human fibular epiphysis variation in relation to mobility patterns, environmental conditions, subsistence economies and cultural behavior to further explore the role of this bone in reconstructing mobility patterns in past populations.

The sample includes 173 individuals from archaeological and modern human skeletal collections spanning Mid-Late Upper Paleolithic to 20th century, and including hunter gatherers, agriculturalists, herders, and post-industrialists. Virtual three-dimensional models of left fibulae were acquired by computer tomography- and laser-scanning. Fibular proximal and distal epiphyseal

morphology was investigated through 3D (semi)landmark based geometric morphometric methods and compared among populations and mobility strategy categories.

Results indicate that the shape of fibular proximal and distal epiphysis tend to separate groups based on their subsistence strategy and chronology. Some fibular traits (e.g., horizontal proximal tibiofibular and tilted distal talofibular articular surfaces, robust interosseous membrane, broad malleolar fossa, projecting m. *biceps femoris*) indicate greater range of mobility of fibular joints and load sharing during ankle and knee excursion in highly active populations. Unique fibular shapes may be therefore associated to a mobile subsistence strategy based on foraging, in a combination of frequent traversing of uneven terrain with no or minimal foot coverings. On the contrary, stiffer range of motions at fibular joints in sedentary populations is consistent with restricted ankle excursion and limited lower limb loading in plain urban settlements while using hard-shoe coverings. Other fibular traits (e.g., shorter subcutaneous triangular surface, laterally projecting malleolus, concave peroneal groove and proximal peroneal insertion) might indicate for mobile groups a more everted foot posture with increased moment arm in eversion, more efficient for barefoot running in forefoot-strike gait and concurring in the stabilization of the first metatarsophalangeal joint. These results further stress the functional role of fibular morphology and its importance in studies investigating past population mobility patterns.

Keywords:

Fibula; Homo sapiens; Subsistence strategy; functional morphology

1. Introduction

Bone cortical and trabecular tissues are considered highly plastic, reacting to the processes of bone modeling and remodeling (Barack, 2020) in response to bone functional adaptation solicited by mechanical loading (Ruff, 2008; Stock, 2018). Although other biological factors (e.g., genetic constraints, bone development, and aging) also play an essential role in the process of bone remodeling (Pearson & Liebermann, 2004; Ruff, Holt, and Trinkaus, 2006), many studies proposed a relationship between an increased mechanical performance of bone and increased strain magnitude, which thus influences overall bone morphology (Ruff, Holt, and Trinkaus, 2006 and reference therein).

Experimental research supports that external bone morphology and structural properties are influenced by variations in the loading regimen, modulated by activity level and mobility (Currey, 1984; Lanyon & Rubin, 1984; Martin & Burr, 1989; Rubin *et al.*, 1990; Lanyon, 1992; Ruff, Holt, and Trinkaus, 2006) and several studies on long bones explored the role of different loading regimens in connection to biomechanical properties and diaphyseal shape (Hagihara & Nara, 2016; Wescott *et al.*, 2014; Sparacello *et al.*, 2014; Haapasalo *et al.*, 2000; Nikander *et al.*, 2006; Shaw and Stock 2009a, 2009b; Trinkaus *et al.*, 1994).

Leg bones' external diaphyseal shape and structural properties were vastly investigated. It was established that high levels of terrestrial mobility are linked to particularly robust cross-sectional geometry and shape at the tibial midshaft (Ruff, 1987; Stock, 2006; Ruff & Hayes, 1983). While tibial robusticity provides information on the level of mobility of a human sample, the tibial diaphyseal shape is also an indicator of loading directionality: haw and Stock revealed that cross-country runners and field hockey players had significantly more robust tibiae than controls, also reflecting different tibial shapes for each group linked to the loading regimen (2009b). The authors further highlighted the correspondence between tibial robusticity in a bioarchaeological sample, fossil hominins, and modern athletes to mobility patterns (Shaw and Stock, 2013). However, mixed evidence resulted from several bioarchaeological studies, which did not find a clear correspondence between tibial shape (I_{max}/I_{min} ratio) and changes in mobility levels (Stock & Pfeiffer, 2001; Holt,

2003; Slad k, Berner and Sailer, 2006; Marchi, 2008; Marchi *et al.*, 2011a; 2011b).

The structural properties and shape of the fibular diaphysis relative to the tibia in response to different loading regimens were also explored. Rantaleinen and colleagues (2010) documented a higher robusticity of the tibia and fibula with high-impact activities (such as..) but also suggested a different performance of the two leg bones, indicating that the tibia and fibula experience different loading responses despite their anatomical vicinity, depending on the amount of fibular weight-bearing. The authors hypothesize that this different response to loading is influenced by the complex interaction of tibiofibular kinematics at both proximal and distal tibiofibular joints (Rantaleinen *et al.*, 2010). Similarly, Marchi and Shaw (2011) documented a progressive increase in fibular robusticity from sedentary controls to runners to field hockey players, possibly linked to a higher degree of ankle dorsiflexion and frequency of cutting movements in the latter groups. Greater ankle medio-lateral excursion modulated the fibula's mechanical load, resulting in higher fibular robusticity relative to the tibia. More recently, Auerbach and coworkers (2017) demonstrated that the cross-sectional properties of the tibia and the fibula vary with respect to their relative position to each other and that directional bending (I_{max} and I_{min}) and average bending rigidity (J) of the fibula is correlated to its distance to the tibia, further supporting the hypothesis that the two bones functions both complementarily and in single units. Overall, the structural properties of the two leg bones respond to and document loading solicitations in terms of intensity and repetitiveness. However, their common interface following mechanical stresses and the link to other variables such as environment and mobility is still the subject of an ongoing investigation. Indeed, several studies considered fibular structural properties among human archaeological samples to explore its role and correspondence to mobility patterns in bioarchaeological contexts, comparing habitual activities of different past populations and from different geographical regions (Marchi, 2008; Sparacello & Marchi, 2008; Marchi, Sparacello and Shaw, 2011; Sparacello *et al.*, 2014; 2018; Hagihara & Nara, 2016). Marchi, Sparacello, and Shaw (2011) examined the impact of mountainous and uneven terrain on the

fibular robusticity of the Italian Neolithic and Iron Age samples compared to medieval and modern populations inhabiting flat geographical areas. They tested the possible effect of habitual and repeated foot eversion/inversion movements, required by habitual weight-bearing activities on uneven terrains, on the mechanical loading of the fibula. The authors revealed that fibular robusticity, either considered alone or paired with the tibia, is correlated with mobility levels and is influenced by uneven terrain (Marchi, Sparacello, and Shaw, 2011). Sparacello, Marchi, and Shaw (2014) further documented higher relative fibular robusticity in a mountain-dwelling archaeological sample compared to samples from regions with flat terrain. According to the authors, this environmental difference indicates frequent directional changes occurring in uneven terrain during the exploitation of inland resources by Upper Palaeolithic hunter-gatherers and Neolithic pastoralists. Sparacello and coworkers (2018) further examined the Mid to Late Upper Paleolithic – Mesolithic transition as documented by structural and shape variations in the tibiofibular complex correlating with mobility levels and types. They showed that individuals chronologically spanning this transition are comparable to modern athletes, documenting their high activity levels.

While the leg diaphyseal shape and cross-sectional geometrical properties have been quite well explored, less is known about the external morphology of their extremities, even though the shape and size of articular facets were found to strongly correlate with joint mobility and habitual movements (Ruff, 1988; Ruff & Runestad, 1992; Ruff *et al.*, 1994; Currey, 2002). However, little investigation has been performed on tibiae and fibulae epiphyseal and talar morphology and articular facets. Regarding the study of tibial and talar epiphyseal shape using human skeletal collections, several investigations adopted a variety of methodological solutions to explore external shape morphologies for a variety of scopes (e.g., Latimer *et al.*, 1987; Stevens & Vidarsdóttir, 2008; DeSilva, 2009; Turley *et al.*, 2011; Frelat *et al.*, 2012; 2017; Sorrentino *et al.*, 2020). A squared talocrural joint shape was found in humans, uniformly distributing forces across the joint in bipedal locomotion with a perpendicular orientation to the long axis of the tibial shaft. In great apes, the joint is trapezoidal with an anterior tilt to accommodate the foot in extreme

dorsiflexion during climbing (DeSilva, 2008; 2009; DeSilva & Throckmorton, 2010; Venkataraman *et al.*, 2013; Latimer *et al.*, 1987; Sorrentino *et al.*, 2020; Turley *et al.*, 2015). While most of these studies focus on morphological comparisons among hominids, Brzobohatá and colleagues (2014) explored variations in the morphologies of tibial extremities of archaeological populations belonging to different socioeconomic living conditions (high vs. low economic status). However, they did not identify a statistically significant correspondence between tibial epiphyseal shape and socioeconomic category. However, a possible trend in distal epiphyseal shape was detected. Indeed, a complex interaction of genetic and derived factors acts on external bone morphology, possibly challenging the reconstruction of finer details of behavior (Lieberman, 1997; Kivell, 2016).

Similarly, Sorrentino and coworkers (2020) study examined the variations in talar morphology in highly mobile hunter-gatherers and more sedentary groups. They revealed that the former group exhibited laterally oriented talar trochleae with a flared lateral malleolar facet, linked to a more everted posture and a more robust and medially oriented talar neck/head, associated with long-distance walking performed barefoot or wearing minimalistic footwear along uneven terrains. On the other hand, sedentary samples show a more parasagittal orientation of the trochlear axis and a less flared lateral malleolar facet linked to a neutral posture and less range of motion at the ankle joint (Barnett & Napier, 1952; DeSilva, 2009; Latimer *et al.*, 1987). Sorrentino and colleagues (2020) concluded that modern human talar shapes vary with locomotor and cultural behavior differences.

Several studies demonstrated that fibular morphology is linked to locomotor behavior in mammals (Barnett & Napier, 1953; Carleton, 1941). In modern humans and primates, the degree of mobility of the fibula has been related to the degree of eversion/inversion and dorsiflexion/plantarflexion at the ankle joint (Marchi *et al.*, 2007). Recent 3D geometric morphometric (3D-GM) studies revealed that proximal and distal fibular morphology are indicators of locomotor patterns associated with each extant hominid taxon (Marchi *et al.*, 2022; Pietrobelli *et al.*, 2023). Pietrobelli & coworkers (2023) documented for proximal epiphysis a more horizontal proximal tibiofibular joint (PTFJ), a

superiorly flat fibular head, an anterolateral and inferior expansion of hamstring and peroneal muscles insertions area onto the fibular head, and a defined but shallower, more medially expanded m. *soleus* insertion in great apes compared to humans. For distal epiphysis, differences are in the orientation of the lateral malleolus, fibulotalar articular facets, and subcutaneous triangular surface (STS) proportions, a wider peroneal groove and a deeper and broader malleolar fossa in great apes than in humans (Marchi et al. 2022). Proximal and distal fibular epiphyseal shapes are, therefore, suggestive and linked to locomotor behavior in living hominids. However, to our knowledge, fibular epiphyseal morphologies were not tested as indicators of mobility and lifestyle among *Homo sapiens* groups to reveal finer locomotor strategies adopted.

Indeed, the transition from foraging to agriculture and finally to urban life, which caused a significant reduction in terrestrial mobility, culminated in highly sedentary lifestyles in modern industrial populations and marking a fundamental cultural, demographic, and behavioral shift (Ruff, 2008; Ruff *et al.*, 2015). Furthermore, it was proposed that these diachronic changes have resulted in bone gracilization and skeletal changes in the shape of modern humans (Ruff *et al.*, 1993), thought to be a consequence of decreased mechanical stimuli resulting in reduced apposition of bone tissue (Chirchir *et al.*, 2015; Ryan & Shaw, 2015; Stock, 2006; Holt, 2003).

In this study, we adopted a 3D-GM approach to explore shape variability of fibular proximal and distal extremities among Italian archaeological populations dated from the Mid-Late Upper Palaeolithic to 20th century, including groups with different subsistence strategies characterized by a progressively declining activity levels spanning from hunter-gatherers, agro-pastoralists to pre- and post-industrialists. Moreover, we explore possible shape variations related to terrain type and shoe use to test fibular shape as a potential indicator of finer cultural aspects.

2. Materials and Methods

2.1.1. Sample description

The analyzed sample consists of human skeletal fibulae belonging to 173 individuals from 21 Italian archaeological sites and osteological collections

either grouped chronologically and geographically, spanning Mid-Late Upper Palaeolithic to the 20th century (Table 1) (Figure 1a). Individuals of both sexes are included, and age-at-death spans between 17-88 years. Sex-related differences in fibular shape were deemed insignificant but primarily due to size (Pietrobelli *et al.*, 2022a). Therefore, males and females were pooled in our analysis.

The Sardinia (SAR) and Emilia-Romagna (ER) sample is part of the Modern Identified Frassetto Collection housed at the University of Bologna. This osteological collection was assembled by Prof. Fabio Frassetto (Belcastro *et al.*, 2017; 2022) and includes individuals from Bologna, Parma, Sassari, Cagliari, and Nuoro, located in Emilia-Romagna and Sardinia (Italy). These sedentary populations include males and females who died during the first two decades of the 20th century (1918–1932) and inhabited the urban area of these cities and their immediate rural surroundings. These individuals are primarily manual laborers, employed in the primary sector either working in the agricultural supply chain (such as in small cheese factories) or employed in small-scale artisanal business (such as in leather factories) (Brigaglia, 2012; Milella *et al.*, 2012; 2015; Belcastro *et al.*, 2017). These jobs were moderately physically demanding but implied a low mobility level, limited within the sedentary context of the developing industrialized economy of a newly urbanized area and its province (Milella *et al.*, 2015). Coherently, it is possible to hypothesize for this sample the use of leather or rubber, hard-soled foot coverings, such as shoes and boots (Sorrentino *et al.*, 2020).

Individuals from Roccapelago (ROC) lived in mountainous (>1000 km above sea level) settings in the Apennines within the Modena province (Italy) between the 17th–18th century. Historical evidence and isotopic studies suggest that the pastoralist subsistence of this population is linked to seasonal transhumance, when shepherds conducted herds to Tuscany, transporting materials and goods for trade (Lugli *et al.*, 2017; Traversari, 2017). This occupation involved high levels of activity, as such activities required constant mobility. Consequently, shepherds preparing for the seasonal transhumance typically wore heavy leather boots with rigid soles nailed to the shoe leather upper (Calzolai, 1998).

Individuals from the Jewish cemetery of via Orfeo (OR) (Bologna, Italy) were unearthed between 2012-2014 by the Superintendence of Archaeology, Fine Arts and Landscape of Bologna, Modena, Reggio-Emilia and Ferrara (SABAP-BO) and date from the late 14th to the mid-16th century (Pini, 1983; Di Stefano *et al.*, 2018; Belcastro *et al.*, 2018; 2019a; Curina & Di Stefano, 2019; Pietrobelli *et al.*, 2020). This population refers to the Jewish community of the Late Medieval - Early Modern Bologna, as it emerged from several archival and cartographical sources (Curina & Di Stefano, 2019). Historical documentation vastly attests that this Bolognese community had sedentary, urban occupational activities, involved in trading primarily as possessors of loan institutions and typographies or as merchants and artisans working on clothing, leather, and jewelry (Todeschini, 2018; Muzzarelli, 2019). This kind of occupation is only modestly physically demanding, with mobility restricted to the typical setting of a small urban town in the Early Modern Age. This population may have utilized soft leather coverings (Zallot, 2018).

Individuals from the early medieval necropolis of Vicenne - Morrione (VCN) are dated to the 7th CE using relative dating methods, such as burial organization, numismatics, and weapon typology (Arslan, 2004; Ceglia, 2010; Ceglia & Marchetta, 2012; Gasparini *et al.*, 2022). These populations refer to a Germanic community that scattered into this hilly landscape centering around an inner plateau in Molise, in the central Italian Apennines, after the collapse of the Western Roman Empire. They carried cultural elements of provincial Roman (Pannonian and Norican) cultures, as well as of nomadic steppe peoples (Avars, Bulgars, Sarmatians, and Proto-Slavs) (Gasparini *et al.*, 2022). This group was characterized by a highly hierarchized and gendered societal organization structured around military activity for males. They partook in high-intensity activity with heavy use of a weapon for defense and territorial control (Gasparini *et al.*, 2022), as also documented by the abundant frequency of interpersonal violence (e.g., skeletal traumatic lesions) among males (Belcastro & Facchini, 2004; Belcastro *et al.*, 2001; Belcastro *et al.*, 2002; Belcastro *et al.*, 2005). Based on historical accounts (Paulus Diaconus, *Historia Langobardorum*) and archaeological parallels (Koncz, 2015) for this population, it is possible to hypothesize the use of soft leather shoes, often completed with

metal buckles and shoe fittings.

Individuals from the Roman necropoles of Suasa (SUA) (Marche, Italy) lived between the 2nd-4th centuries AD in the urban settlement of Suasa, established during the 3rd century BC in a key hilly location to manage the Roman territories from the coast to the Apennine valleys after the Roman expansion into the area. This population is characterized by a high level of permanence in the urban settlement with deficient mobility, as attested by the chronologically continuous occupation and expansion of the town and funerary areas (Giorgi, 2012). Epigraphical sources for this settlement attest to occupational activities mentioning a collegium *fabrii et centonarii*, a professional corporation in metal and clothing processing but also employed in fire brigades (Antolini, 2000). However, the settlement mainly relies on agricultural activities, attested by the acquisition of productive land by illustrious families, and on the production of either building or domestic materials (Antolini, 2000). Footwear included a variety of soft leather shoes and sandals with flexible leather soles, typically worn barefoot or with socks (Goldman, 1994).

The population of Casalecchio - Ceretolo (CER) refers to a Boian Celtic community dating to the 4th-3rd century BC and settled into the hilly river Reno valley, in the proximity of the Etruscan center of Felsina, the city of Bologna (Italy) (Ortalli, 1995). The necropolis, discovered and excavated in 1991-1992, comprises 96 inhumations with 100 individuals and hosted a population referring to the "La Tène" culture, encompassing exclusively Celtic cultural elements (Mariotti *et al.*, 2005; Brasili *et al.*, 2000), with little to no influence of the Etruscan-Italic components, conversely typical of other surrounding Celtic settlements in the same area on the Apennines fluvial valleys (Vitali, 1992; Vitali, 1987). Based on the location of the selected individuals within the funerary area, pertaining to its early utilization (first half of 4th century BC), it is possible to hypothesize that such individuals belonged to Celtic populations who migrated into this landscape at the time, with a limited degree of admixture with local Italic communities (Belcastro *et al.*, 2010). Societal organization, centered on a tribal community, structures upon military activity, as also attested by the burials of warriors in this context,

carrying weapons. At the same time, tombs of females had personal ornaments, indicating gendered divisions (Ortalli, 1995). Based on coeval findings relating to similar groups in the surroundings, this population also benefitted from a subsistence based on agriculture, arboriculture, and farming of pigs and sheep (Grassi, 1991). The use of soft leather shoes, usually worn with wool socks, is documented by archaeological findings of Celtic cultures (Quillien, Harlow, Michel, 2020).

The population of Olmo di Nogara (ODN) belonged to a community of the "Terramare," settled into the Northern Padania alluvial plain near the river Po valley of Western Veneto (Cavazzuti *et al.*, 2019) during an advanced phase of Middle Bronze 2 (MB2) and Late Bronze Age (BR2) (Salzani, 2005; Canci *et al.*, 2015; Pulcini, 2014). We do not have direct evidence of these settlements. However, during the initial phases of the Middle Bronze Age, this kind of site was typically characterized by small settlements (typically 1–2 ha), with estimated 125/130 inhabitants per ha confined within substantial fortifications, later progressively enlarged to over 10 ha hosting 1000 individuals or more (Cardarelli, 2015; Sabatini *et al.*, 2018). The necropolis of Olmo di Nogara was excavated between 1987 and 2009 and comprised ~ 533 burials (471 inhumations and 62 incinerations) (Canci *et al.*, 2015). The individuals in this study span the chronology of BM2 and BM3 (1550-1300 BC), as established by burial typology, stratigraphy, and goods, and belong to both 'B' and 'C' areas. All male individuals in this study had swords/daggers as grave goods (Salzani, 2005; De Marinis & Salzani, 2005). This population was structured in a conic clan of tribal characterization, articulated in structured parental ties and with strong warfare connotations and cantered upon military élites and their activities (Cupitò & Leonardi, 2005a; 2005b). Biomechanical stress and palaeopathological indicators, documented in the anthropological study of these individuals, revealed that this population engaged in a physically demanding activity (Canci *et al.*, 2015; Cecchetto, 2020; Pulcini, 2014; Canci *et al.*, 2005), often connected to warfare (Canci *et al.*, 2009; Beck Di Lotto, 2010). Besides military activity, during these phases, hunting, fishing, agropastoralism, metallurgy, and textile production are documented in Northern Italy (Cocchi Genick, 2009). Based on archeological evidence, it is

possible to hypothesize the use of textiles (wool, animal skins, vegetal fibers) for cloth and shoe production (Sabatini *et al.*, 2018; Harris, 2007).

The Neolithic remains (NEO) belong to a sample from four caves (Arene Candide, Arma dell'Aquila, Grotta Pollera, and Bergeggi) near Finale Ligure in Liguria (Italy) (Marchi *et al.*, 2006, 2011; Marchi, 2008; Sparacello & Marchi, 2008; Sparacello *et al.*, 2011, 2014; 2019; Varalli *et al.*, 2020). These sites were the object of early archaeological explorations since the mid-1800s. However, the archaeological, anthropological, and chronological study for the rational reorganization and characterization of this skeletal series was only recently undertaken, revealing a radiocarbon dating chronology spanning 5209-4364 BCE, comprising both cultural attributions of Square Mouthed Pottery period and Impresso-Cardial Complex (Sparacello *et al.*, 2018; 2019 and references therein). The locations of sites are enclosed within a mountainous area with very rugged terrain (Sparacello *et al.*, 2014). Neolithic subsistence in the area relied on pastoralism and the exploitation of terrestrial resources, likely during periodic transhumance (Macphail *et al.*, 1997; Rowley-Conwy, 1997; Marchi *et al.*, 2011), which also benefitted from the uneven terrain of the area and natural system of caves which contributed to controlling animals (Maggi & Nisbet, 2000; Marchi *et al.*, 2011). Animal skins to fabricate cloths and utilized for producing shoes are vastly attested in the Middle Neolithic Square-mouthed Pottery culture of Northern Italy (Harris, 2014; Starnini & Voytek, 1997a). Although the use of animal skin shoes is not directly documented in this context, microwear patterns on stone and bone tools (including awls) at Arene Candide are indicative since they are considered compatible with the preparation and treatment of soft materials, likely animal skins for cloth making (Maggi *et al.* 1997; Starnini & Voytek, 1997a; Starnini & Voytek, 1997b; Harris, 2014). Animal resources for such productions include wild and domesticated *Bos* sp., *Capra* and *Ovis*, *Cervus elaphus*, and *Capreolus capreolus* (Rowley-Conwy, 1997). In addition, other Middle Neolithic contexts in Northern Italy suggest evidence of basketry, which could also be employed for cloth and shoe making. However, no such traces were detected at Arene Candide (Harris, 2014). Suggestive is the attire of the chronologically similar "Iceman" (5300 BP) from the Alpine Italian region, the oldest direct finding of

a shoe in the territory, which comprises soles fabricated with *Ursus arctos* skin, padded with grass, uppers made with *Cervus elaphus* skin and sustained with a netting of lime bast (Groenman-van Waateringe, 1993; Wood, 1999; Harris, 2007; O'Sullivan *et al.*, 2016).

The Upper Palaeolithic-Mesolithic sample (UP) groups includes individuals found in funerary contexts of the Italian peninsula, from the Ligurian caves of Barma Grande (Barma Grande 2, Barma Grande 5, Barma Grande 6; Gravettian primary depositions, ~25.000-24.000 BP; Formicola, 1988; 1989; Formicola *et al.*, 2004;) and Arene Candide (Arene Candide 2, Arene Candide 3, Arene Candide 4, Arene Candide 12; Epigravettian primary and secondary depositions: ~10.700 and ~10.000 BP; Formicola *et al.*, 2005; Sparacello *et al.*, 2018, Macphail *et al.*, 1994), the Sicilian caves of San Teodoro (San Teodoro 1; Epigravettian primary deposition, ~14.000-10.000 BP (14.800 BP for San Teodoro 1); Fabbri *et al.*, 1993; D'Amore *et al.*, 2009), Grotta dell'Uzzo (Uzzo 1A, Uzzo 1B, Uzzo 2, Uzzo 5; Mesolithic primary depositions; ~9.500-8.500 BP; Borgognini Tarli and Repetto, 1985; 1986; Borgognini Tarli, 1993), and Grotta d'Oriente (Oriente B; Mesolithic primary deposition, ~10.500 BP, Mannino *et al.*, 2012; Di Salvo *et al.*, 2012; Catalano *et al.*, 2020) and the shelters of Mezzocorona-Borgonuovo (Sauveterrian primary deposition – radiocarbon dating refer to an early Neolithic period of ~6.500-6.000 BP, in discordance with the Mesolithic archaeological evidence; Dalmieri *et al.*, 1998; 2002; Gazzoni *et al.*, 2021) and Mondeval de Sora (Castelnovian primary deposition; ~7.500 BP Alciati *et al.*, 1992; Fontana 2006; Fontana *et al.*, 2016; 2020; Gazzoni *et al.*, 2021) located in Trentino. Subsistence strategy for Late Upper Palaeolithic-Mesolithic individuals varied based on the exploitation of different resources, comprising either terrestrial (e.g., animal and plants) or occasional marine/freshwater elements. It was generally based on hunter-gathering and foraging, which included high-level activities based on seasonal availability (Mannino *et al.*, 2015; Gazzoni *et al.*, 2021; Sparacello *et al.*, 2018), albeit with a decreased high-mobility task involving walking and running in comparison to Mid Upper Palaeolithic (Holt, 1999; 2003). Sparacello and colleagues (2018) suggested that these chronological groups were both highly mobile and shared a similarly high level of loads on the lower limb. Moreover, all these sites are

characterized by an elevation typical of mountainous settings and moderate to marked terrain ruggedness (Sparacello *et al.*, 2014; 2020; Gazzoni *et al.*, 2021). The use of shoes among Mid Upper Palaeolithic and Mesolithic hunter-gatherers is debated (Sorrentino *et al.*, 2020), as there is material evidence of barefoot footprints in chronologically similar contexts (Pastoors & Lenssen-Erz, 2021). However, the use of soft footwear could not be excluded, as also attested by the use of soft shoes based on ichnological evidence (Ledoux *et al.*, 2021) and foot morphological considerations (Trinkaus, 2005).

Based on historical sources, archaeological information, ethnographic accounts, and landscape data outlined above, we classified each population according to subsistence strategy (hunter-gatherers, agro-pastoralists, and sedentary pre- and post-industrials), terrain typology (flat, hilly, and mountainous; adapted following the protocol of Sparacello and colleagues (2014), but considering the categories of “moderately hilly” and “hilly-mountainous” as a single one, here named “hilly”,) and possible shoe use (hard-sole shoes soft-sole shoes and barefoot) (Figure 1b).

2.1.2. Digital acquisition

SAR and a subset of ER 3D surface models were obtained through computed tomography (CT), using a Revolution Discovery CT dual energy at Istituto Ortopedico Rizzoli (Bologna, Italy) (slice thickness and increment at 0.625 mm, voltage 100 kV, X-ray tube current 360 mA, reconstruction at 40 keV with "Detail" filter, voxel size ranging from 0.390 to 0.507 x 0.625 mm). The reconstructed DICOM (16-bit gray-scale, signed) images were then processed with Avizo 9.2 (Thermo Fisher Scientific) for image segmentation with half-maximum height (HMH) protocol outlined by Spoor and coworkers (1993) following Coleman and Colbert (2007). An isosurface was then generated for each segmentation.

ROC, OR, VCN, SUA, CER, and ODN samples were digitized with ARTEC Space Spider 3D (Luxembourg), housed at the BONES Lab in the Department of Cultural Heritage of the University of Bologna, a mobile and structured light laser scanner (point-accuracy: 0.05 mm; mesh resolution: 0.10 mm). The acquisition took place in two recording sessions for each specimen, then integrated with the scanner software (Artec Studio 9) after first cleaning and

manual alignment. These aligned scans were globally registered. A single polygonal 3D mesh was created with the Sharp Fusion algorithm, finally generating the 3D surface model exported in .stl (Little Endian) format (Artec Group, 2013).

NEO and UP-MESO samples were digitized using the structured light scanner DAVID® SLS-2 (DAVID Group 2007-2015).

2.2. 3D Geometric morphometric analysis

A template of 142 landmarks and (semi)landmarks was created in Viewbox v. 4.0, capturing both fibular extremities, and covering major muscle, ligament, and tendon attachment sites and articular surfaces (Figure 2, Table 2). The template configuration was applied to all target samples in Viewbox v. 4.0. Semilandmarks were allowed to slide on curves and surfaces, minimizing thin-plate spline (TPS) bending energy between the targets and the template (Slice, 2006) so that semilandmarks could be considered geometrically homologous among specimens (Gunz, Mitteroecker & Bookstein, 2006; Gunz & Mitteroecker, 2013). The template repeatability (i.e., high intra-observer agreement) and applicability to 3D models generated with different scanning devices were previously assessed by Pietrobelli *et al.* (2022).

Raw Cartesian coordinates were imported in R (version 4.0.3) (R Core Team, 2020), and a further sliding of semilandmarks was performed against recursive updates of the Procrustes consensus while computing two Procrustes superimpositions via Generalized Procrustes Analysis (GPA), one GPA for each epiphysis after sub-setting the respective coordinates accordingly. A further GPA was carried out only on distal talofibular surfaces as previously studies have suggested its variation in humans with different subsistence strategies (Sorrentino *et al.*, 2020). Each set of raw coordinates, separated for each epiphysis and for distal talofibular surfaces, were thus converted into standardized, scaled, centered, and oriented shape coordinates (i.e., Procrustes coordinates) (Slice, 2006; Rohlf & Slice, 1990) using the `gpgen()` function in the R package "geomorph" version 3.3.2 (Adams *et al.*, 2018). Outliers from Procrustes consensus were found utilizing the function `plotOutliers()` provided in this package and removed. The centroid size (CS) was also calculated and used as a proxy of the size of both fibular extremities

separately (Slice, 2006).

Procrustes coordinates for each epiphysis were then subjected to a Principal Component Analysis (PCA) to explore shape variations among populations and categories of subsistence strategy, terrain type, and shoe use (Schlager, 2017). Procrustes ANOVA was utilized to evaluate shape differences among different populations and categories of subsistence strategy, terrain type, and shoe use, calculating Procrustes distances among specimens using a residual randomization procedure (RRPP = T, iterations = 1,000), with the R package "geomorph" v. 3.3.2 (Adams *et al.*, 2018).

3. Results

3.1. Proximal epiphysis

Figure 3 shows the three-dimensional shape space PCA scatterplot of each population mean according to chronology along the first three PC and extreme shape variations along the first two PC. The first three PCs combined account for 45.48% of the total variance explained (PC1: 17.67%; PC2: 15.57%; PC3: 12.24%). Despite some overlapping, the plot shows a trend in separating populations according to chronology, considering the extremes, along the first two principal axes. Except for ROC, most historic populations fall within positive PC1 and PC2 scores. On the other hand, UP and CER lay within PC2 negative scores, while NEO and ODN fall within PC1 negative scores. Significant shape differences among populations are revealed by Procrustes ANOVA comparisons (Table 3), which shows that modern populations (SAR, ER, and OR) significantly differ from earlier populations (UP, NEO, and ODN). Interestingly, the two modern, sedentary populations of SAR and ER differ significantly from each other, and similarly, UP and NEO populations significantly differ. Populations with low-intensity activity tending to plot toward PC1 positive and PC2 positive are characterized by an oblique and diminutive PTFJ, a raised styloid process, and less bulky insertions of the biceps femoris tendon and *m. peroneus longus*. Highly active and with an earlier chronology populations possess a horizontal and wide PTFJ, an absent styloid process, and projecting insertions (most detected by PC1 negative and PC2 negative).

When population means are grouped by subsistence strategy, terrain type,

and shoe use categories (Figure 4), it is possible to detect a pattern determining variations among sedentary populations with low activity subsistence, living in flat terrain and using hard-sole shoes in comparison to other categories. Procrustes ANOVA comparisons among categories confirm this pattern, with sedentary populations differing significantly from either agriculturalists and hunter-gatherers, populations living in flat terrain differing significantly from those living in mountainous areas, and populations using shoes differing significantly from those possibly not using any (Table 4-6). However, some outlier are present for each category: CER is grouped outside the range for all categories and ODN is grouped outside the range of mountain-dwellers and soft-shoe users (Figure 4).

Centroid size comparisons (Figure 5) reveal little to no variation related to size (ANOVA: p -value > 0.05).

3.2. Distal epiphysis

Figure 6 shows the three-dimensional shape space PCA scatterplot of each population mean according to chronology along the first three PC and extreme shape variations along the first two PC. The first three combined PCs account for 57,12% of the variance explained (PC1: 27.32%; PC2: 20.01%; PC3: 9.79%). Despite some overlapping, the plot shows a trend in separating populations according to chronology along PC2. Significant shape differences among populations are confirmed by Procrustes ANOVA comparisons (Table 3), which shows that modern populations (SAR, ER, and OR) significantly differ from earlier populations (UP, NEO, and ODN). Similarly to proximal epiphysis, for distal epiphysis, comparisons among modern SAR and ER show a significant difference, making comparisons among UP, NEO, and ODN. In addition, ROC shows a significant difference from ER.

Most historic populations fall within or towards positive PC2 scores, except for VCN. On the other hand, UP, NEO, ODN, and CER lay within PC2 negative scores. Prehistoric and protohistoric populations with high-intensity activity show that the talofibular articular surfaces are anteroposteriorly broad, with a voluminous malleolus that is more anteriorly oriented in inferior view, a shortened STS and a robust ILA insertion with marked rugosity. At the same time, the malleolar fossa and the peroneal groove are enlarged and more

concave. On the other hand, historic and modern populations with low-intensity activity show narrow talofibular articular surfaces, a diminutive malleolus laterally oriented in inferior view, an elongated STS, and a less robust – even though elongated – ILA insertion and narrow and less concave malleolar fossa and peroneal groove.

When landmarks and semilandmarks covering only the talofibular articular surfaces are isolated and subjected to a separate additional GPA, with a PCA showing a similar distribution pattern among populations separating highly active and sedentary populations along either PC1 and PC2 (SI Figure 1) extreme shape reconstruction highlight an additional shape difference among active and sedentary populations concerning the orientation of the proximal and distal talofibular articular surfaces (PAF and DAF, respectively). In prehistoric and protohistoric populations with high-intensity activity, the DAF is markedly more prominent than the PAF (with a markedly oblique superior margin) and highly flared and downward-facing. In contrast, in historic and modern populations with low-intensity activity, PAF (with a straight superior margin) and DAF have a similar vertical orientation (SI Figure 1).

Similarly, When population means are grouped by subsistence strategy, terrain type, and shoe use categories (Figure 4), we highlighted a pattern determining variations among sedentary populations with low activity subsistence, living in flat terrain, and using hard-sole shoes in comparison to other categories. Procrustes ANOVA comparisons among categories confirm this pattern, with sedentary populations differing significantly from either agriculturalists and hunter-gatherers, populations living in flat terrain differing significantly from those living in mountainous areas, and populations using shoes differing significantly from those possibly not using any (Table 4-6). However, some outlier are present for each category: ROC is grouped outside the range of mountain-dwellers and OR falls outside the range of soft-shoe users (Figure 4). Centroid size comparisons (Figure 5) reveal slight variation related to size emerging in pairwise comparison only between OR and UP (p -value <0.05).

4. Discussion

In this study, we highlighted shape changes of fibular proximal and distal ends

among Italian archaeological populations dated from the Mid-Late Upper Palaeolithic to the 20th century through a 3D-GM approach. We found a pattern consistent with a progressive decline of activity level associated with the type of terrain and shoe use, especially evident when comparing hunter-gatherers to sedentary pre- and post-industrialists (Figure 3-6; Table 4-6). Our results agree with previous evaluations of proximal and distal fibular morphology (Marchi, 2015; Marchi *et al.*, 2022; Stern & Susman, 1983; Pietrobelli *et al.*, 2022; Ogden, 1974a; 1974b) and add on the functional role of the fibula and its potential in inferring mobility patterns in modern humans (Sparacello *et al.*, 2018). Our results are also consistent with those obtained on fibular structural properties among human archaeological samples supporting the role of this bone in differentiating mobility patterns in bioarchaeological contexts, comparing habitual activities of different past populations and from different geographical regions (Marchi, 2008; Sparacello & Marchi, 2008; Marchi, Sparacello and Shaw, 2011; Sparacello *et al.*, 2014; 2018; Hagihara & Nara, 2016). Furthermore, we documented significant shape variations among the most and least active groups in the orientations of proximal and distal tibiofibular articular surfaces and ligament and tendon insertion robusticity. These morphological changes likely reflect biomechanical differences in response to locomotor behavior among these groups, referring to the specific knee (Frelat *et al.*, 2017; Sylvester, 2013; Sylvester & Pfisterer, 2012; Tardieu, 1999) and ankle (Barnett & Napier, 1953; DeSilva, 2009; Latimer *et al.*, 1987; Stern & Susman, 1983) postures. However, when comparing different populations with intermediate levels of mobility, a less clear pattern appears, suggesting a complex interaction of regular lower limb loading regimen and other environmental and cultural variables.

Regarding shape changes documented in the proximal epiphysis, the PTFJ is diminutive and obliquely oriented in historic populations with low-intensity activity. On the other hand, highly active, earlier populations possess a horizontal and wide PTFJ (Figure 3). The PTFJ in humans shifts posterolaterally as the ankle dorsiflexes (Barnett & Napier, 1953; Odgen, 1974a; 1974b). The orientation of the PTFJ surface may range from 5° to 80° of inclination (Eichenblat & Nathan, 1983). An angled PTFJ is linked to the requirement for

bipedal locomotion (Susman & Stern, 1983; Pietrobelli *et al.*, 2023). Ogden (1974a; 1974b) found an association among oblique joints ($>20^\circ$) with smaller articular surface areas and decreasing mobility, as the joints have a reduced ability to accommodate torsional forces, with a higher incidence (70%) of subluxation or dislocation. In contrast, the less inclined PTFJ enables greater mobility at the joint and is, therefore, less prone to injury (Alves-da-Silva *et al.*, 2019). Therefore, sedentary humans with an angled PTFJ are linked to a less mobile tibiofibular articulation at the knee (Eichenblat & Nathan, 1983; Odgen, 1974a; 1974b; Sarma *et al.*, 2015) and associated with a reduced dorsiflexion axis inclination at the ankle (Barnett & Napier, 1953; Odgen, 1974a; 1974b). Indeed, humans with a sedentary lifestyle likely have a habitual neutral posture and less range of motion at the ankle joint (Barnett & Napier, 1952; DeSilva, 2009; Latimer *et al.*, 1987; Parr, Chatterjee, & Soligo, 2012; Sorrentino *et al.*, 2020). On the other hand, hunter-gatherers with high intensity running and walking in uneven terrain (Figure 4; Table 5) show morphological features at the ankle (i.e., trapezoidal talocrural joint) that allow a broader range of ankle excursion in the parasagittal plane (dorsal and plantar flexion) (Latimer *et al.*, 1987). (Oygucu, Kurt, Ikiz, Erem, & Davies, 1998; Trinkaus, 1975). Moreover, a horizontal PFTJ, as seen in highly active populations, would provide greater lateral knee stabilization through greater joint mobility in knee flexion (Chang *et al.*, 2020; Huang *et al.*, 2021), but ultimately leading to lateral knee osteoarthritis (OA) (Pietrobelli *et al.*, 2023), as also attested by the higher incidence of OA in prehistoric populations compared to agriculturalist (Bridges, 1989; 1991; Knusel, 1996).

Historic populations also possess a raised styloid process, while in highly active populations, the styloid process is markedly less pronounced (Figure 3). A marked styloid process surmounting the fibular head sustains the lateral compartment of the knee in full extension (Tardieu, 1999), as it is the attachment for the popliteus tendon through the popliteofibular ligament (Aiello & Dean, 1990). In addition, the bony protuberance of the styloid process, associated with these soft structures at the posterolateral corner of the knee, prevents posterior translation, varus angulation, and primary external rotation of the tibia relative to the femur (LaPrade *et al.*, 2001; 2003).

Conversely, highly mobile hunter-gatherers, which travel large daily distances and home ranges (average 9.5 km for females and 14.1 km for males – modern hunter-gatherers: Marlowe, 2005) and have an equally convenient energetic cost of barefoot running both in the forefoot and rearfoot strike (Cunningham *et al.*, 2010), may be facilitated by a more mobile PTFJ in knee flexion during barefoot running, as increased knee flexion is more frequently attained (Hall *et al.*, 2013). This link between barefoot running and facilitated knee flexion, thanks to the more mobile PTFJ in mobile groups, may also explain the increased robusticity and width of the m. *biceps femoris* tendon insertion on the fibula (Figure 3; Table 6). Experimental studies on Jomon hunter-gatherers revealed that this population had a posteriorly elongated linea aspera, the site of femoral insertion of the m. *biceps femoris*, suggesting its greater mass and possible use increase (Mizushima *et al.*, 2015).

Lastly, in highly active populations, the m. *peroneus longus* proximal insertion is more concave and medially projecting. Peroneal muscles activate in the support phase of locomotion (Jungers, Meldrum & Stern, 1993; Stern & Susman, 1983), everting the inverted foot and laterally stabilizing the inverted ankle joint while traversing a medially inclined ramp (Bavdek *et al.*, 2018).

This mechanism may be crucial for hunter-gatherers traversing rugged terrains and further supports the results of Sparacello and coworkers (2014; 2018), which documented an increase in fibular robusticity with frequent directional changes occurring in uneven terrain of Upper Palaeolithic hunter-gatherers and Neolithic pastoralists. In addition, this result is comparable to those obtained for the morphology and trabecular organization of the talus, reflecting an adaptation to uneven terrain that increases mediolateral movement, thus requiring higher stability (Carlson *et al.*, 2007; Sorrentino *et al.*, 2020). Sorrentino and colleagues (2020) also linked hunter-gatherers' more flexible talar configuration to barefoot/soft shoe support, in contrast to individuals from post-industrial societies whose use of heavy leather shoes and boots may limit ankle excursions in eversion and inversion. This finding seems comparable to our results, where barefoot and hard-shoe-using populations are very distinctly distributed (Figure 4; Table 6).

As regards shape changes documented in the distal epiphysis, prehistoric and

protohistoric populations with high-intensity activity have anteroposteriorly broad talofibular articular surfaces, with a markedly larger DAF, also highly flared and downward facing than the PAF. In contrast, PAF and DAF have a similar vertical orientation in historic and modern populations with low-intensity activity (Figure 6; SI Figure 1). This result is in agreement with a hypothesized increased vertical component of the ground reaction force transmitted by the fibula through the talus in the ankle joint in mobile populations compared to sedentary ones, in parallel with observations in hominids (Stern and Susman, 1983, but see also Latimer *et al.*, 1987) and consistent with an increased degree of ankle dorsiflexion (Barnett & Napier, 1953; DeSilva, 2009; Marchi, 2015; Marchi *et al.*, 2022). In addition, differences in the more oblique superior margin of PAF seen in hunter-gatherers compared to the straight margin seen in sedentary populations were also associated with a more plantarflexed ankle configuration (Stern & Susman, 1983). Comparably, this feature in great apes would suggest their greater ankle joint mobility than humans (Marchi *et al.*, 2022). This fibular morphology, with larger, highly flared, and downward facing DAF with a superior oblique margin, may reflect a more "flexible" shape (i.e., a shape configuration sustaining greater talocrural joint motion range) as suggested by Sorrentino and colleagues (2020), compatible with a more everted ankle posture of hunter-gatherers. Historic, sedentary populations, in comparison, have medially facing, vertical PAF and DAF, which may suggest a more "stable" shape (i.e., indicating limited talocrural joint motion range) referring to a more neutrally loading ankle (Sorrentino *et al.*, 2020).

Interestingly, the results of Sorrentino and colleagues (2020) document the presence of markedly flared lateral malleolar facets in the tali of hunter-gatherers, directly mirroring the downward-facing DAF of hunter-gatherers observed in our study. According to Sorrentino and coworkers (2020), in addition, the stable, static configuration of the ankle, compatible with the vertical, medially oriented PAF and DAF in sedentary populations, is compatible with a constraint in motion range using heavy leather shoes and boots (Figure 4; Table 6). While these results are backed by theoretical studies on the loading patterns at the ankle (Preuschoft, 1970), as well as empirical

studies conducted on the fibula/tibia diaphyseal robusticity (Marchi, 2007, 2015b; Marchi *et al.*, 2019), a recent study (Venkataraman *et al.*, 2013), however, could not identify any significant morphological changes of the habitual dorsiflexion of the foot in modern hunter-gatherers. Therefore, this mixed evidence suggests overall caution in this interpretation. Experimental kinetic and kinematic studies are needed to measure the impact of different ranges of motions with activity/terrain/shoe use among modern hunter-gatherers and controls on the morphology of the ankle bones.

Prehistoric and protohistoric populations with high-intensity activity also show shorter STS with a voluminous, projecting malleolus that is more anteriorly oriented in the inferior view. On the other hand, historic and modern populations with low-intensity activity show elongated STS with a diminutive malleolus laterally oriented in an inferior view (Figure 6). A shorter STS in great apes than humans was previously linked to their more extended peroneal muscle bellies and shorter peroneal tendons (Marchi *et al.*, 2022), which contribute to more potent and more precise eversion/inversion movements, as it is generally accomplished in mammals (Biewener, 2016). A similar muscle-tendon unit dynamic in humans could hypothetically reflect a powerful inversion/eversion and plantarflexion of the foot. In addition, it could support the more everted ankle joint posture that our result on STS and malleolus shapes may indicate for mobile groups (Sorrentino *et al.*, 2020).

Furthermore, we may suggest that the projecting morphology and anterior orientation of the malleolus with a shortened protruding STS in mobile groups may provide an extended moment arm for the peroneal muscles in eversion (Hinterman *et al.*, 1994), as their tendinous part (i.e., the peroneal tendon, comprising the mm. *peroneus longus* and *brevis*) runs immediately posterior to this bony structure, partially covering it (Morgan *et al.*, 2020). The peroneal tendon is one of the strongest everting structures, while the foot is plantarflexed (Hinterman *et al.*, 1994). Given that the forefoot-strike pattern in barefoot endurance running, which among other factors, was linked to unshod human ancestors and modern groups (Hatala *et al.*, 2013 and references therein), provides a greater degree of foot plantarflexion (Hall *et al.*, 2013; Almeida *et al.*, 2015), we may tentatively suggest that in mobile

populations a raised morphology of the malleolus with a conjoined shorter and anteriorly oriented STS in beneficial. Thanks to the positioning of the evertors' tendon and its moment arm, this morphology may facilitate a powerful eversion. At the same time, the foot is plantarflexed and lateral ankle stabilization and involved in a unique biomechanical strategy for ground reaction force dissipation and injury risk mitigation (Liebermann *et al.*, 2010).

Conversely, an elongated STS with a less protruding malleolus in sedentary groups may be linked to a diminished peroneals' eversion function in both plantarflexion and dorsiflexion, with relevant implications for the injury mechanism of lateral ankle sprains (Morgan *et al.*, 2020). Connected to this hypothesized biomechanical dynamic for the peroneal tendon, we observed an enlarged, more concave peroneal groove hosting the peroneal tendon in mobile populations. In contrast, in sedentary populations, peroneal grooves are narrower and flatter (Figure 6). The shape of the peroneal groove was evaluated in clinical studies, documenting that convex and flat grooves are directly associated with peroneal tendon dislocation/subluxation (Mabit *et al.*, 1999; Cozzetto *et al.*, 2014). This result would further strengthen the hypothesis of the reduced ankle mobility in sedentary groups due to narrower peroneal grooves, as on the contrary, the surgical deepening of this sulcus would provide a reduced pressure between the tendon-groove interface at different foot orientations (Title *et al.*, 2005; Morgan *et al.*, 2020). Following this interpretation, a thinner retromalleolar area might suggest a thinner peroneal muscle with reduced muscle mass, as mentioned by Marchi and colleagues (2022). A thinner peroneus longus is implicated in a complex locking process of the first metatarsal against the medial cuneiform in individuals with pronating feet (Angin *et al.*, 2014; Morgan *et al.*, 2020). We may suggest that a thinner peroneal groove may be involved in a reduced medial stabilization of the first metatarsal-phalangeal ray of the foot in resisting motion by pulling it into plantarflexion (Morgan *et al.*, 2020). Indeed, it was demonstrated that after transecting the peroneus longus, a considerable medial displacement of the transverse arch might occur (Bohne *et al.*, 1997). The wider peroneal groove observed in mobile populations could be, therefore, consistent with a greater need for stabilization caused by an increased medial deviation of the

first metatarsal in barefoot individuals and lateral deviation of the hallux in shod feet (Barnett, 1962; Trinkaus, 2005; Zipfel & Berger, 2007). Indeed, studies describing the leg's muscular and tendinous structure (e.g., Marchi *et al.*, 2018) with bone morphology associated with locomotor behavior in humans are needed to assess this hypothesis further.

A robust ILA insertion with marked rugosity characterizes mobile populations, while a less robust – even though elongated – ILA insertion is shown in sedentary populations (Figure 6). This site contributes to the tibiofibular syndesmosis with the interosseous membrane attachment site through the interosseous ligament (Hermans *et al.*, 2010). Given its function as a buffer, neutralizing forces during the heel-strike phase in walking and stabilizing the talocrural joint during loading, we suggest that its greater enthesal robusticity in mobile populations may play a critical role in the load-sharing ability of the fibula relative to the tibia during loading (Skraba *et al.*, 1984) and traversing uneven terrains (Sparacello *et al.*, 2014; 2018) (Table 5).

The malleolar fossa of mobile populations is broader and more concave, while in sedentary populations, it is less expanded and shallower (Figure 6). This site provides insertion of the posterior tibiofibular ligament, which stabilizes the talocrural joint, prevents posterior talar translation, and dissipates internal rotation forces in the ankle joint (Ebraheim *et al.*, 2006; Golanó *et al.*, 2010; Hermans *et al.*, 2010; Perrich *et al.*, 2009); it also hosts the posterior/transverse talofibular ligaments, which restrict internal and external rotation, talar tilt, and dorsiflexion (Rasmussen *et al.*, 1983). The spreading of the malleoli stretches these ligaments as they excurse over the wedge-shaped talar trochlea in ankle dorsiflexion (Barnett & Napier, 1952; Close & Inman, 1952; Hicks, 1953; Trinkhaus, 1975). Sorrentino and colleagues (2020) found an expanded anterior mediolateral margin of the talar trochlea in hunter-gatherers and associated this feature with passive dorsiflexion extremes such as in frequent and habitual squatting. Thus, we may propose that the enlargement of the malleolar fossa in mobile populations may reflect a possible elongation of the ankle collateral ligaments and frequent tightness with prolonged pressure, such as in squatting (Viidik, 1966; Trinkaus, 1975). Similarly, a wider malleolar fossa in great apes was indeed associated with

larger collateral ligaments in great apes due to the higher frequency and magnitude of dorsiflexion experienced during climbing (Marchi *et al.*, 2022).

Populations with intermediate levels of mobility clustered variably (Figures 3-6). In the case of the proximal epiphysis, the 17th-18th population of Roccapelago clustered towards Neolithic and Bronze Age individuals. This result may be congruent considering a similarly intense activity due to subsistence strategy based on seasonal transhumance in Roccapelago and Neolithic populations (Marchi *et al.*, 2011; Sparacello *et al.*, 2018; Lugli *et al.*, 2017) and to the similar high physical demands that the agriculturalists/warriors of Olmo di Nogara may have sustained (Cohen, 1977). All these three populations may have shared the necessity of dissipating tensile forces on the lower limb and accommodating the PTFJ excursion in knee and ankle flexion (Barnett & Napier, 1953; Odgen, 1974). At the same time, a different pattern emerges for Roccapelago in the distal epiphysis, therefore either this hypothesized dynamic favoring knee and ankle flexion may manifest differently depending on specific joint (proximal vs. distal end) biomechanics, similar to what was observed for the tibia (Frelat *et al.*, 2017), or other factors (e.g., ancestry) than those examined played a part, suggesting for the proximal epiphysis a more complex pattern when populations in-between hunter-gathering and most sedentary extremes are compared.

Similarly, there is a significant difference in the proximal fibular shape of Neolithic populations and hunter-gatherers of older chronology (Table 3), despite equal geography and comparable habitual high activity level: Karim and colleagues (1981) showed that agricultural labor creates the same level of aerobic fitness of physically trained soldiers. Therefore, similar shapes among the two groups could be expected. However, while the progressive distribution of along PC2 for distal epiphysis shows a more straightforward gradient with activity level (Figure 6), still individuals from Olmo di Nogara differ from hunter-gatherers, and Neolithic populations have a significantly different shape (Table 3), and the population of Vicenne tends to cluster towards active populations. Again, this latter issue might be either explained by the peculiar social structure of this population, heavily focusing on physically demanding military activity or by other variables such as ancestry influencing bone

morphology: indeed, the populations of Vicenne is of Avar ancestry (Gasparini *et al.*, 2022). This interpretation may also be hypothesized for the population of Ceretolo (Ortalli *et al.*, 1995), clustering towards active populations in both proximal and distal ends, as these populations may have trained and participated in combat actions and had Celtic ancestry.

Nevertheless, neither of these groups has significant differences from the most and least active extremes, similar to other populations with intermediate mobility (Table 3). Differences among modern, post-industrial populations also emerged (Table 3). Skeletal comparisons among the Sardinian and Northern Italian population of Emilia-Romagna already revealed occasional inter-population variability in tibial and fibular measurements (Calzoni, 2021), the timing of skeletal maturation (Belcastro *et al.*, 2019b), sexual dimorphism (unpublished data). Indeed, the reason for this microscale variation in fibular morphology may lie within the genetic diversity of Sardinia and Emilia Romagna and the emergence of unique regional-based traits (Chiang *et al.*, 2018; Pelotti *et al.*, 2008).

Another potential explanation for the morphological differences is related to age changes. For example, human peroneal muscles have also been reported to form concave furrows along the anterior fibular surface of the diaphysis in correlation with a combined effect of age and mechanical loading (Chevalier & Tignères, 2020; Hagihara & Nara, 2016). However, further studies using a broader sample are needed to assess whether other factors, such as skeletal development, age, diet, ancestry, and climate, could affect fibular shape (Betti *et al.*, 2014).

A possible limitation of this study is the fact that our categories of subsistence, terrain and shoe use often overlap in the same population. For instance, the most mobile population of hunter-gatherers from the Mid-Late Upper Palaeolithic is also likely barefoot and mountain-dwelling. Therefore, to better discern the influence of every single variable under study, including modern hunter-gatherers with more diverse environmental conditions (e.g., Venkataraman *et al.*, 2013), would be beneficial.

5. Conclusions

In conclusion, the morphological investigation of fibular proximal and distal

ends among Italian populations dated from the Late Upper Palaeolithic to the 20th century through a 3D-GM approach revealed shape changes that are consistent with a decline of activity level in conjoint action of the type of terrain and shoe use, especially evident when comparing hunter-gatherers to sedentary pre- and post-industrialists. In addition, some fibular traits (e.g., horizontal proximal tibiofibular and tilted distal talofibular articular surfaces, robust interosseous membrane attachment, broad malleolar fossa, projecting m. *biceps femoris*) indicate greater mobility of fibular joints and degree of load sharing by the fibula during an anteroposterior excursion of the knee and ankle in highly active populations, compared to more rigid fibular joints in sedentary populations. These morphologies may be related to habitual and frequent ankle and knee flexion that is consistent with their foraging subsistence strategy in a combination of frequent traversing of uneven terrain with no foot coverings in the former and restricted ankle excursion by limited lower limb loading and use in plain urban settlements with hard-shoe coverings in the latter. Other fibular traits (e.g., shorter STS, projecting malleolus, concave peroneal groove, and proximal insertion) might indicate for mobile groups a more everted foot posture with increased moment arm in eversion, more efficient for barefoot running in forefoot-strike gait and concurring in the stabilization of the first metatarsophalangeal ray. Overall, these results reveal the potential of fibular morphology as an indicator of locomotion behavior and mobility patterns, further reporting on anatomical correlates to the role of physical activity in bone functional adaptation.

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Data availability statements

Raw coordinates, sample lists and R scripts used for this analysis are available upon request addressed to the corresponding author.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

AP conceptualization, investigation, formal analysis, validation, writing-original draft, methodology, writing-review, and editing; RS investigation, formal analysis, validation, writing-original draft, methodology, writing-review, and editing; VS validation, writing-original draft, methodology, writing-review, and editing; DM, SB, MGB conceptualization, supervision of the research, methodology, project administration, software, writing-review and editing.

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Tables

Population	Chronology	Subsistence	Terrain	Shoe use	F	M	ND	T
SAR (Sassari, Nuoro, Cagliari)	Contemporary Age (late 19 th - early 20 th century CE)	sedentary/post-industrial	flat/hilly	hard-soled	26	41	-	67
ER (Bologna, Parma)	Contemporary Age (late 19 th - early 20 th century CE)	sedentary/post-industrial	flat	hard-soled	19	18	-	37
ROC (Roccapelago)	Modern Age (17 th -18 th century CE)	agriculturalists/herders	mountainous	hard-soled	-	-	10	10
OR (via Orfeo)	Early Modern Age (14 th -16 th century CE)	pre-industrial, sedentary agriculturalists	flat	soft-soled	4	4	-	8
VCN (Vicenne)	Early Middle Ages (7 th -8 th century CE)	agriculturalists/military activity	hilly	soft-soled	2	2	-	4
SUA (Suasa)	Roman Late Imperial Age (1 st -3 rd century CE)	agriculturalists	hilly	soft-soled	5	3	-	8
CER (Casalecchio Ceretolo)	Second Iron Age (4 th -3 th century BC)	agriculturalists/military activity	hilly	soft-soled	-	2	-	2
ODN (Olmo di Nogara)	Middle Bronze Age (1500-1300 BC)	agriculturalists/military activity	flat	soft-soled	2	5	-	7
NEO (Arene Candide, Grotta Pollera, Arma dell'Aquila, Bergeggi)	Neolithic (6000-4500 BCE)	agriculturalists/herders	mountainous	soft-soled	3	10	2	15
UP (Arene Candide, Barma Grande, Grotta dell'Uzzo, San Teodoro, Grotta d'Oriente, Mezzocorona, Mondeval de Sora)	Mid Upper Palaeolithic-Mesolithic (24.000-7500 BP)	hunter-gatherers/fish foragers	mountainous	barefoot/soft-soled	4	11	-	15
Total					65	96	12	173

Table 1. Sample composition by population, chronology and sex.

Landmarks	Definition
L1	Point where the <i>fibular anterolateral border</i> divides into two ridges: the proximal apex of the <i>subcutaneous triangular surface</i> (STS)
L2	Most medial point of the medial border of the STS
L3	Most lateral point of the lateral border of the STS
L4	Most distal point of the <i>lateral malleolus</i> in anterior view
L5	Most distal point of the posterior border of the <i>malleolar fossa</i>
L7	Most anterior point on the anterior border of the <i>proximal fibular-talar articular facet</i> (PAF)
L8	Point between the anterior border of PAF and the <i>anterior border of distal fibular-talar articular facet</i> (DAF)
L9	Most distal point of DAF
L10	Most proximal point on the posterior border of DAF
L12	Most posterior point of the proximal border of PAF
L13	Most proximal point of <i>proximal tibio-fibular articular facet</i>
L14	Most proximal point of <i>interosseous tibio-fibular ligament</i> (ILA) insertion
L15	Most proximal point on styloid process of fibular head in medial view
L16	Most antero-proximal point on anterior border in medial view (above fibular neck)
L17	Most postero-proximal point on posteromedial border in medial view (above fibular neck)
L18	Most postero-proximal point on posterior border in lateral view (above fibular neck)
Curves	Definition
C_1->2	Medial border of the <i>subcutaneous triangular surface</i> (STS)
C_1->3	Posterior border of the STS
C_7->8	Anterior border of the <i>proximal fibular-talar articular facet</i> (PAF)
C_8->9	Anterior border of the <i>distal fibular-talar articular facet</i> (DAF)
C_9->10	Posterior border of the DAF

	Number of semi-landmarks
C_1->2	5
C_1->3	7
C_7->8	1
C_8->9	1
C_9->10	1

C_10->12	Posterior border of the PAF	1
C_8->10	Border between the PAF and DAF	1
C_13->13	Outline of <i>proximal tibio-fibular articular facet</i>	6
C_7->12	Proximal border of the PAF	2
Surfaces	Definition	Number of semi-landmarks
SSML_malleolar fossa	Surface of the <i>malleolar fossa</i> , attachment site of the <i>transverse tibiofibular</i> and <i>posterior talofibular ligaments</i> .	7
SSML_ILA	Attachment surface of <i>interosseous tibio-fibular ligament</i> and part of <i>interosseous membrane</i> (ILA)	13
SSML_fibular groove	Groove for tendons of m. <i>peroneus longus</i> and m. <i>tertius</i> and attachment site of <i>posterior tibiofibular ligament</i> .	13
SSML_STS	<i>Subcutaneous triangular surface</i> (STS)	24
SSML_FiTal1Ar	Proximal <i>fibular-talar articular facet</i> (PAF)	4
SSML_FiTal2Ar	Distal <i>fibular-talar articular facet</i> (DAF)	3
SSML_head	Proximal <i>tibio-fibular articular surface</i>	5
SSML_prox_ep	Surface of <i>proximal epiphysis</i>	32

Table 2. Landmark and semilandmark template configuration for fibular proximal and distal epiphysis. For each landmark and semilandmark, name, definition and number are reported. See Figure 2 for visual display and landmark positioning on the fibular ends.

<i>Procrustes ANOVA pairwise comparisons: Populations</i>											
<i>prox</i>	<i>dist</i>	SAR	ER	ROC	OR	VCN	SUA	CER	ODN	NEO	UP
	SAR	-	0.001	ns	ns	ns	ns	ns	0.036	0.001	0.001
	ER	0.004	-	0.014	ns	ns	ns	ns	ns	0.002	0.005
	ROC	ns	ns	-	ns	ns	ns	ns	ns	ns	ns
	OR	ns	ns	ns	-	0.031	ns	ns	0.022	0.003	0.002
	VCN	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
	SUA	ns	ns	ns	ns	ns	-	ns	ns	ns	ns
	CER	ns	ns	ns	ns	ns	ns	-	ns	ns	ns
	ODN	ns	0.052	ns	ns	ns	ns	ns	-	0.010	0.009
	NEO	0.007	0.001	ns	0.022	ns	ns	ns	ns	-	ns
	UP	0.012	0.049	ns	0.028	ns	ns	ns	ns	0.039	-

Table 3. Results of pairwise comparisons after Procrustes ANOVA test among populations, showing in bold significant shape differences. Below the diagonal are displayed comparisons for proximal epiphysis, while above the diagonal are displayed comparisons for distal epiphysis. ns= not significant. See Table 1 for abbreviations used for populations

<i>Procrustes ANOVA pairwise comparisons: Subsistence</i>				
<i>prox</i>	<i>dist</i>	sedentary	agriculturalist	hunter-gatherers
	sedentary	-	0.001	0.001
	agriculturalist	0.003	-	ns
	hunter-gatherers	0.025	ns	-

Table 4. Results of pairwise comparisons after Procrustes ANOVA test among subsistence categories, showing in bold significant shape differences. Below the diagonal are displayed comparisons for proximal epiphysis, while above the diagonal are displayed comparisons for distal epiphysis. Ns= not significant.

<i>Procrustes ANOVA pairwise comparisons: Terrain</i>				
<i>prox</i>	<i>dist</i>	<i>flat</i>	<i>hilly</i>	<i>mountainous</i>
	<i>flat</i>	-	ns	0.001
	<i>hilly</i>	ns	-	ns
	<i>mountainous</i>	0.001	ns	-

Table 5. Results of pairwise comparisons after Procrustes ANOVA test among terrain tipology categories, showing in bold significant shape differences. Below the diagonal are displayed comparisons for proximal epiphysis, while above the diagonal are displayed comparisons for distal epiphysis. Ns= not significant.

<i>Procrustes ANOVA pairwise comparisons: Shoe use</i>				
<i>prox</i>	<i>dist</i>	<i>hard shoes</i>	<i>soft shoes</i>	<i>barefoot</i>
	<i>hard shoes</i>	-	ns	0.001
	<i>soft shoes</i>	ns	-	0.005
	<i>barefoot</i>	0.036	0.027	-

Table 6. Results of pairwise comparisons after Procrustes ANOVA test among shoe use categories, showing in bold significant shape differences. Below the diagonal are displayed comparisons for proximal epiphysis, while above the diagonal are displayed comparisons for distal epiphysis. Ns= not significant.

Figures

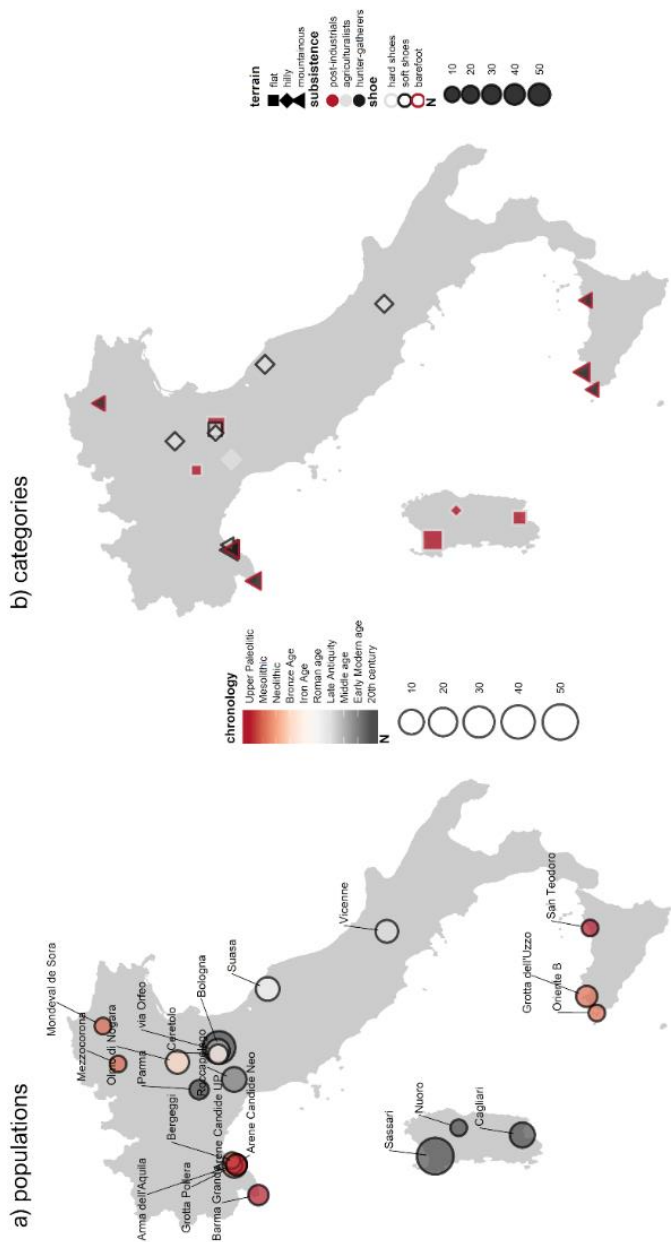


Figure 1a. The archeological samples included in this study with respective provenance location, chronology and numerosity. **1b.** the samples according to categories of subsistence (fill), terrain (shape) and shoe use (stroke).

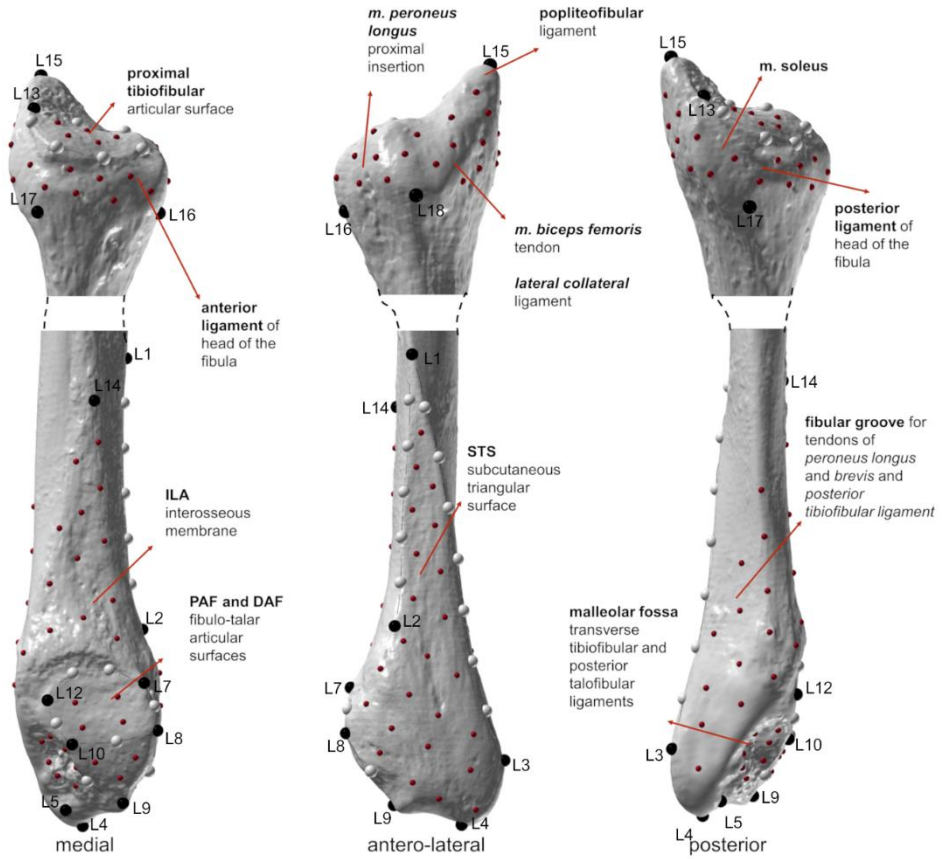


Figure 2. The template configuration on a human left proximal fibula in medial view (left), antero-lateral view (centre) and posterior view (right). Black dots are fixed landmarks, grey dots are curve semilandmarks and red dots are surface semilandmarks. See Table 2 for definition of numbered landmarks and curve.

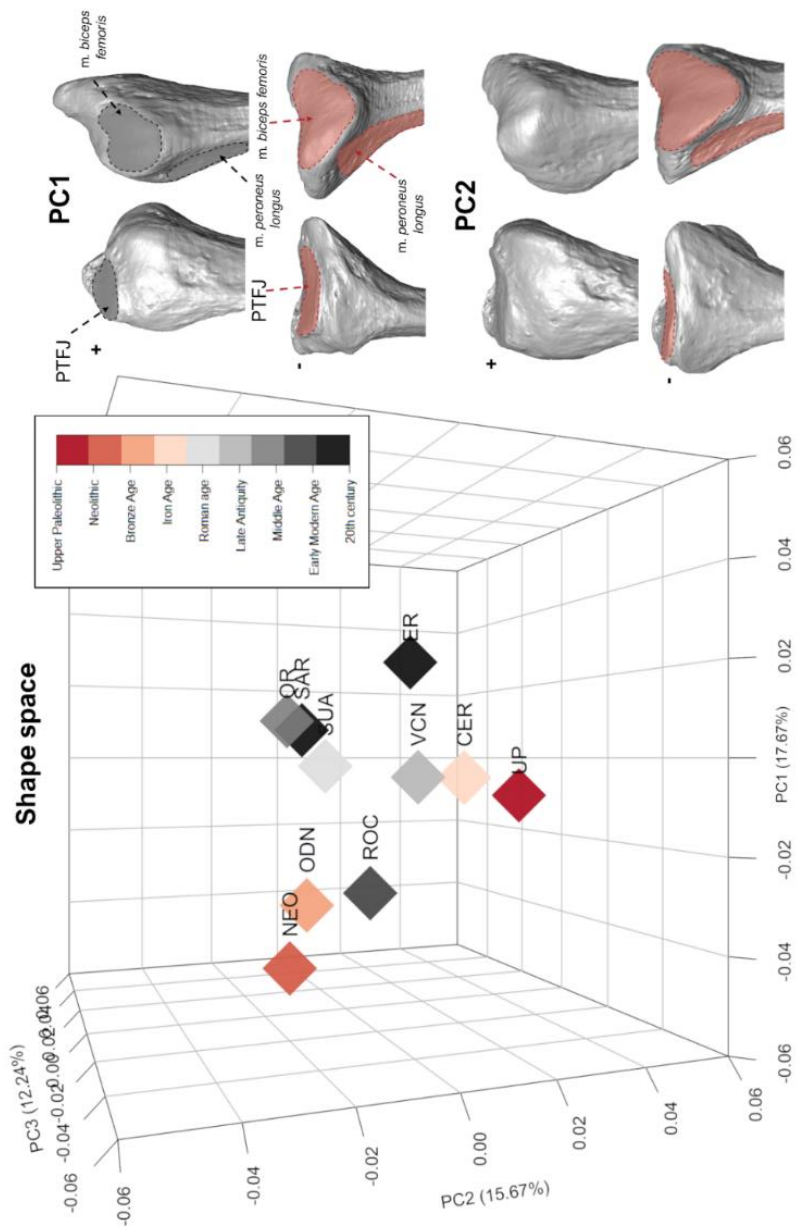


Figure 3. Shape space PCA plot computed for proximal fibular epiphysis (left). Mean shape (rhombus) for each population is displayed in the PCA plot. Extremes shapes warped on fibula model for PC1 (top right) and PC2 (bottom right) positive (+) and negative (-) scores; Morphologies highlighted in black represent features of sedentary, less mobile, recent populations; Morphologies highlighted in red represent features of mobile, highly active, oldest populations. UP= Mid-Upper Palaeolithic/Mesolithic populations; NEO= Neolithic populations; ODN=Bronze Age population of Olmo di Nogara; CER= Iron Age population of Casalecchio-Ceretolo; SUA= Roman population of Suasa; VCN= Late-Antiquity population of Vicenne; OR= Middle Age-Early Modern Jewish population of via Orfeo cemetery, Bologna; ROC= Late Modern population of Roccapelago; ER= contemporary 20th century population of Emilia-Romagna; SAR= contemporary 20th century population of Sardinia.

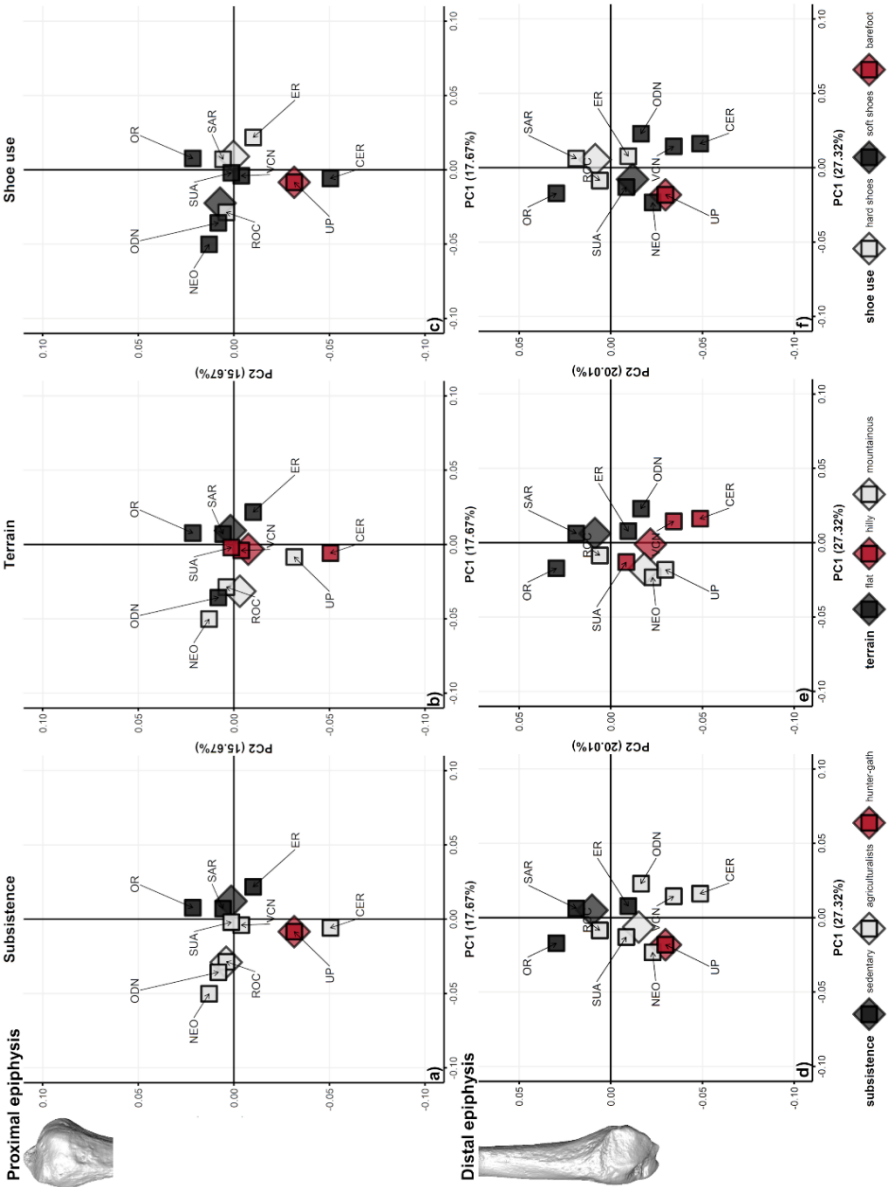


Figure 4. Shape PCA plots for proximal (top) and distal (bottom) fibular configurations displaying the distribution of each population (squares) represented according to subsistence (a;d), terrain typology (b;e) and shoe use (c;f) categories. Mean shape of each category (rhombus) is also depicted. UP= Mid-Upper Palaeolithic/Mesolithic populations; NEO= Neolithic populations; ODN=Bronze Age population of Olmo di Nogara; CER= Iron Age population of Casalecchio-Ceretolo; SUA= Roman population of Suasa; VCN= Late-Antiquity population of Vicenne; OR= Middle Age-Early Modern Jewish population of via Orfeo cemetery, Bologna; ROC= Late Modern population of Roccapelago; ER= contemporary 20th century population of Emilia-Romagna; SAR= contemporary 20th century population of Sardinia.

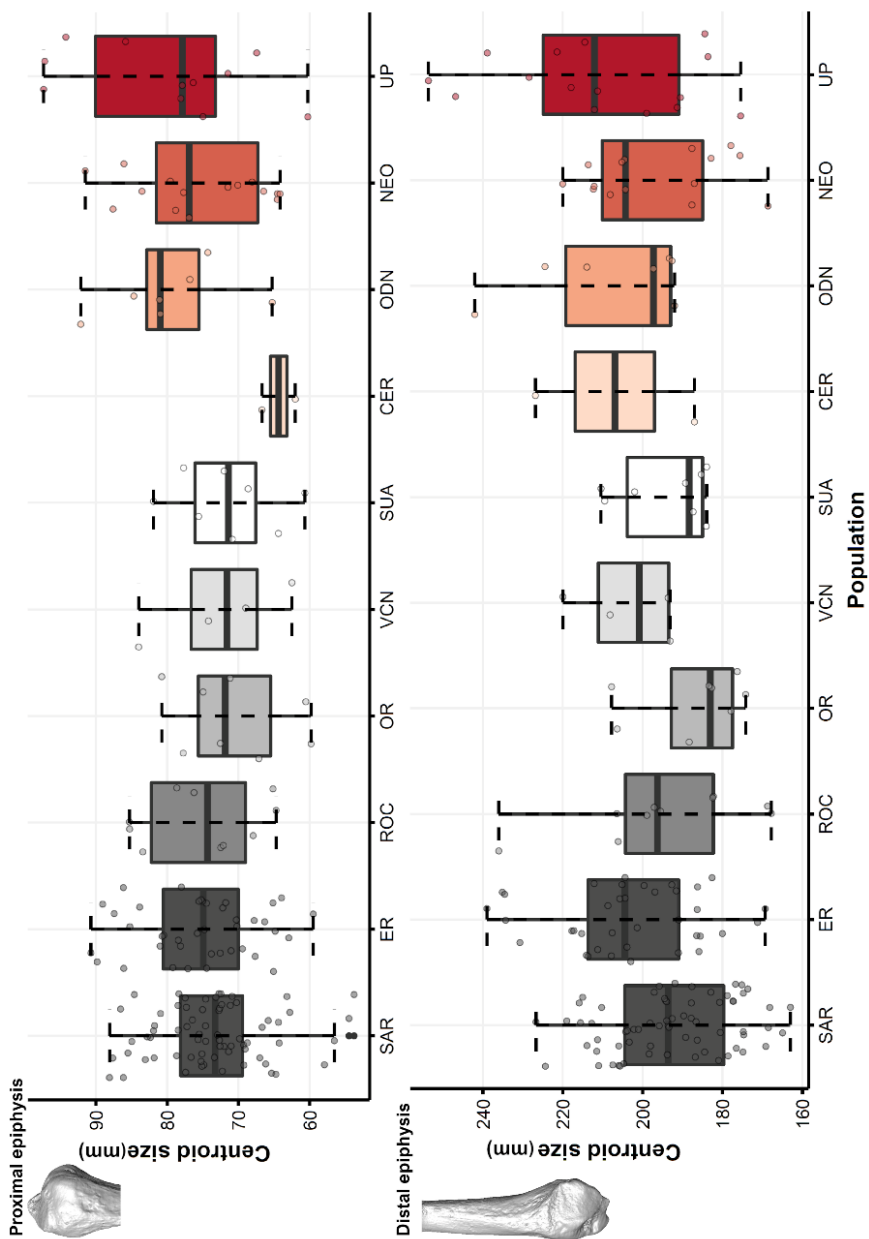


Figure 5. Boxplots for proximal (top) and distal (bottom) centroid size (mm) comparisons. Black lines are the medians, black boxes represent the interquartile ranges, whiskers the non-outliers range and black circles the outliers

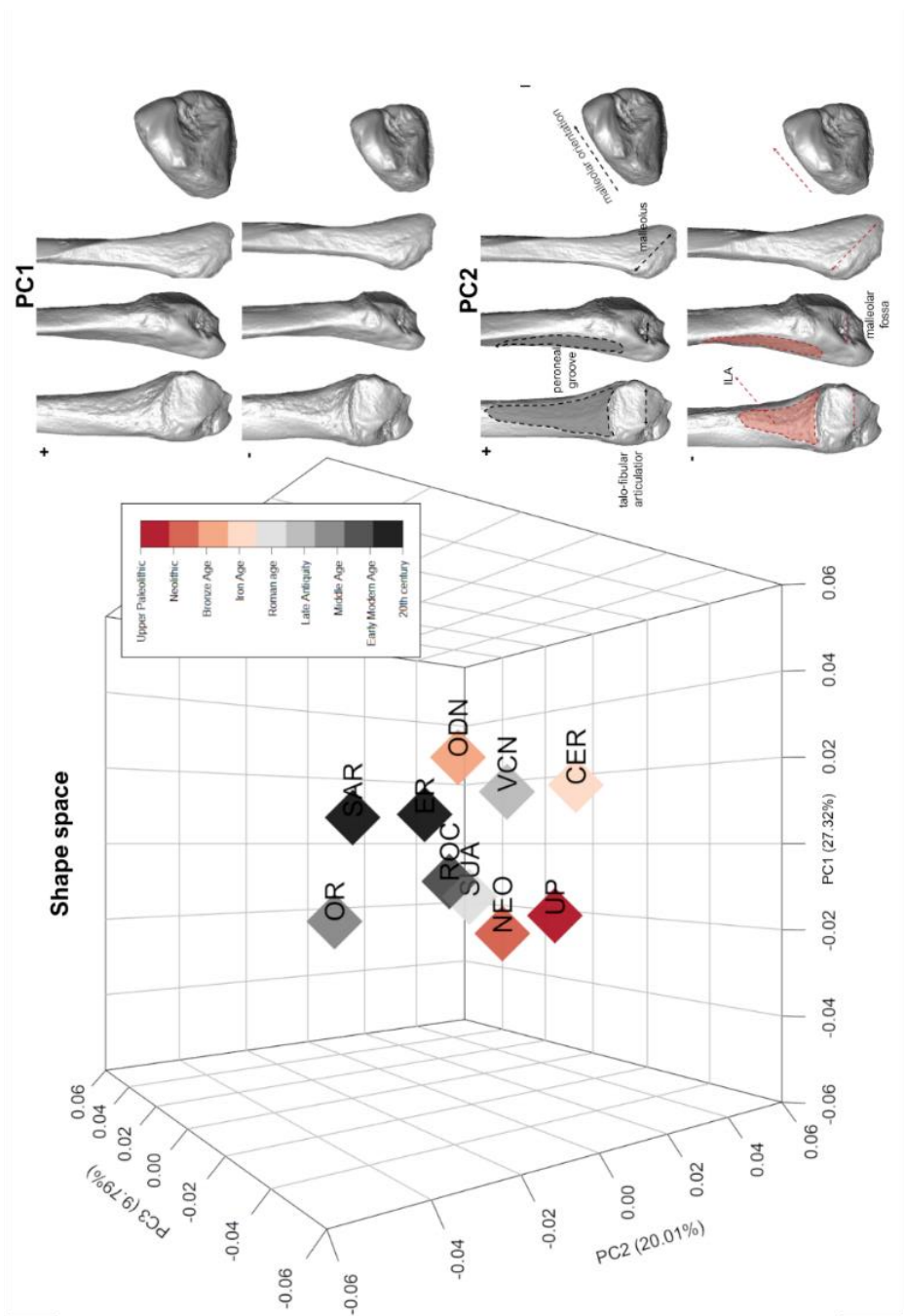
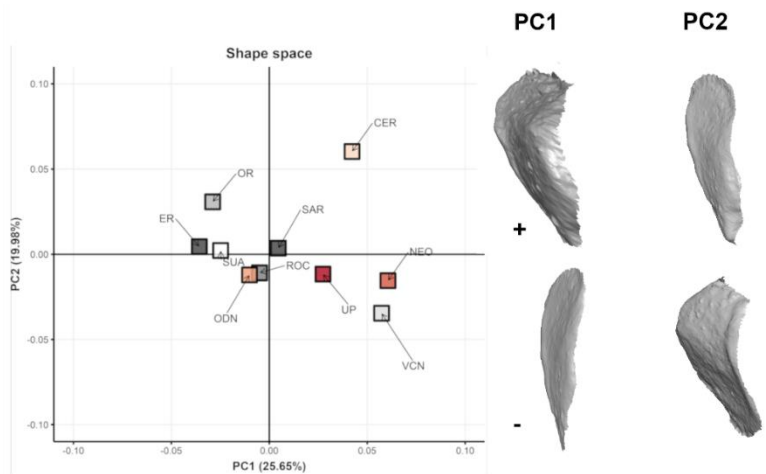


Figure 6. Shape space PCA plot computed for distal fibular epiphysis (left). Mean shape (rhombus) for each population is displayed in the PCA plot. Extremes shapes warped on fibula model for PC1 (top right) and PC2 (bottom right) positive (+) and negative (-) scores; Morphologies highlighted in black represent features of sedentary, less mobile, recent populations; Morphologies highlighted in red represent features of mobile, highly active, oldest populations. UP= Mid-Upper Palaeolithic/Mesolithic populations; NEO= Neolithic populations; ODN=Bronze Age population of Olmo di Nogara; CER= Iron Age population of Casalecchio-Ceretolo; SUA= Roman population of Suasa; VCN= Late-Antiquity population of Vicenne; OR= Middle Age-Early Modern Jewish population of via Orfeo cemetery, Bologna; ROC= Late Modern population of Roccapelago; ER= contemporary 20th century population of Emilia-Romagna; SAR= contemporary 20th century population of Sardinia.

Supplementary material



SI Figure 1. Shape space PCA plot for population means distribution obtained considering distal talofibular landmarks only (left). Extremes shapes (right) warped on a fibula model for PC1 and PC2 positive (+) and negative (-) scores computed considering only landmarks and semilandmarks of proximal and distal fibulotalar articular surfaces (PAF and DAF). Morphologies referring to PC1 negative and PC2 positive extremes represent features of vertically oriented, medially facing articulations of sedentary that characterize less mobile, recent populations. Morphologies referring to PC2 positive and PC2 negative extremes represent features of inferiorly facing, titled articulations of mobile, highly active, oldest populations. Fibulae are in anterior view.



PAPER III

Interspecific variation: locomotion in extant hominids

CONTENTS

Pietrobelli A, Sorrentino R, Benazzi S, Belcastro MG, Marchi D (2023), Linking the proximal tibiofibular joint to hominid locomotion: a morphometric study on extant species. *American Journal of Biological Anthropology*, (10.1002/ajpa.24696)



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Linking the proximal tibiofibular joint to hominid locomotion: A morphometric study of extant species

Annalisa Pietrobelli , Rita Sorrentino, Stefano Benazzi, Maria Giovanna Belcastro, Damiano Marchi

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Title

Linking the proximal tibiofibular joint to hominid locomotion: a morphometric study on extant species

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Abstract

Objectives We perform a comparative assessment of shape variation of the proximal fibula in extant humans and great apes, intending to investigate the possible link between proximal fibular shape and locomotory patterns.

Methods Our sample includes 94 fibulae of 37 *Homo sapiens*, 15 *Gorilla*; 17 *Pongo*; and 25 *Pan*. Fibular morphology was investigated through three-dimensional (semi)landmark-based geometric morphometric methods.

Results We found unique features of the human fibular head compared to that of great apes (i.e., oblique articular surface, the presence of the styloid process, specific morphology of muscle attachment sites), supporting the functional role of this bone in relation to human obligate bipedalism. Great apes also showed distinctive traits in their proximal fibula morphology, in agreement with differences in locomotor behavior.

Conclusion The morphology of the proximal fibula in extant humans and great apes is indicative of locomotor behavior, offering the potential for the comparative analysis of fossil hominin remains.

Keywords:

Fibula; Bipedalism; 3D Geometric Morphometrics; Great apes; Functional morphology

1. Introduction

Obligate bipedalism and upright posture are considered characteristic traits of *H. sapiens*, which is a fully terrestrial biped (Aiello & Dean, 1990; Harcourt-Smith, 2015; Harcourt-Smith & Aiello, 2004). On the other hand, *Pan* and *Gorilla* frequently engage in terrestrial quadrupedalism (~85-95% of their locomotor behavior; Carlson, 2005; Doran, 1996), mostly knuckle-walking/running. Less than ~1.5% of *Pan* locomotor and postural repertoire is dedicated to terrestrial/arboreal bipedalism (Carlson, 2005; Crompton, Sellers & Thorpe, 2010). *Pan* genus engages in frequent (~8-18%) arboreal locomotion (Carlson, 2005; Doran, 1993, 1996), with ~56% arboreal positional behaviors dedicated to vertical climbing/descent (Crompton *et al.*, 2010) and ~11-44% dedicated to arboreal quadrupedalism (Doran, 1996). Indeed, most studies agree that gorillas are less arboreal than chimpanzees and bonobos (Crompton *et al.*, 2010; Doran, 1996; Tuttle & Watts, 1985). Mountain gorillas are usually considered the least arboreal of all the great apes since they primarily engage in terrestrial quadrupedalism (Remis, 1995). They spend less than 1% of total locomotor time engaging in vertical climbing (avoiding terminal branches and in closeness to the center of the tree), mainly performed by females and juveniles (Remis, 1995; Tuttle & Watts, 1985). Both mountain and lowland gorillas engage in terrestrial and arboreal scrambling (Remis, 1995; Tuttle & Watts, 1985). In contrast to mountain gorillas, lowland gorillas may be considered more arboreal since their reported first sightings occurred in arboreal settings in ~ 40% of total sightings. This may be regarded as a proxy indicator of total arboreal behavior (Hunt, 2004; Remis, 1995, 1998). Gorillas also engage in either terrestrial or arboreal bipedal locomotion (1-6%, Crompton *et al.*, 2010), with lowland gorillas performing ~9% of bipedal bouts during arboreal locomotion (Remis, 1995), comparably to mountain gorillas, which in contrast are more engaged in terrestrial bipedalism (Tuttle & Watts, 1985). Orangutan locomotor activity is mainly restricted to the canopy (Thorpe

& Crompton, 2006). Although slight differences in positional behaviors among the species of orangutans are present, the kind of arboreal locomotion employed by the two species does not vary much. The locomotor behavior of this taxon typically involves forelimb suspension with the trunk in orthograde posture and the hindlimb loaded in compression (35%) (Thorpe & Crompton, 2006) and vertical climbing/descent (~25-33%) (Cant, 1987; Manduelli *et al.*, 2012; Thorpe & Crompton, 2006). Orangutans also engage in bipedalism more frequently (7%) than other nonhuman great apes in arboreal settings and with extended hindlimbs (Thorpe *et al.*, 2007). Various morphological traits in the lower limb are associated with bipedalism in humans, among which derived knee, and ankle joints that effectively stabilize the leg and foot during walking are hallmarks (DeSilva, 2008, 2009; Javois *et al.*, 2009; Sylvester & Pfisterer, 2012; Sylvester, 2013; Tardieu, 1999).

The traits of the human knee mainly associated with bipedalism are the *valgus* position, a mechanism for patellar retention due to the elevation of the lateral condylar lip, anteroposterior elongation, and changes in the form of both tibial and femoral condyles that provide tibial dominance and an increased patellar moment arm, and an increased tibial cartilage contact derived by both genomic and epigenetic mechanisms (Frelat *et al.*, 2017; Javois *et al.*, 2009; Lovejoy *et al.*, 2007; Tardieu, 1981; 1999). By contrast, great apes show abducted knees and asymmetrical femoral and tibial condyles with a relatively expanded medial condyle, transmitting more load through the medial compartment of the knee and an increased knee joint mobility determined by reduced femoral-tibial contact (Frelat *et al.*, 2017; Sylvester & Pfisterer, 2012; Sylvester, 2013; Tardieu, 1999).

The human talocrural joint has a squared shape, which uniformly distributes forces across the joint, while in great apes, the joint is trapezoidal to accommodate the foot in extreme dorsiflexion during climbing (DeSilva, 2008; 2009; 2010; DeSilva & Throckmorton, 2010; Venkataraman *et al.*, 2013). The talocrural joint faces inferiorly in modern humans, roughly perpendicular to the long axis of the tibial shaft, with an anterior tilt of the talocrural surface that allows a considerable degree of dorsiflexion that is required during bipedal locomotion (DeSilva, 2009; Latimer *et al.*, 1987; Sorrentino *et al.*, 2020;

Turley, White & Frost, 2015). Previous studies of the knee and ankle joints focused mainly on the femur, tibia, and talus (e.g., Frelat *et al.*, 2017; Harmon, 2006; 2007; 2009; Sorrentino *et al.*, 2020) while the fibula has been generally overlooked. However, several studies have shown that fibular morphology is linked to locomotor behavior in mammals (Barnett & Napier, 1953; Carleton, 1941). Furthermore, in modern humans and primates, the degree of mobility of the fibula has been related to the degree of eversion/inversion and dorsiflexion/plantarflexion at the ankle joint (Barnett & Napier, 1953). Among the works focusing on the lower limb in humans and fossil hominins of Mary W. Marzke (e.g., Marzke, Longhill & Rasmussen, 1988; Nagano *et al.*, 2005), to whom this Special Issue is dedicated, the study of McLean and Marzke (1994) was the first to report variations in fibular cortical thickness among chimpanzees and humans. In addition, recent studies conducted on the relative robusticity (as evidenced by cross-sectional geometry analysis) of the human and nonhuman primate fibula found a relationship between fibula/tibia diaphyseal strength ratios and degree of arboreal locomotion. More arboreal species were characterized by relatively more robust fibulae than terrestrial ones (Marchi, 2007; 2015a). It was proposed that this relationship may reflect the different degree of mobility of the fibula at the ankle joint due to the different range of foot dorsiflexion/plantarflexion and inversion/eversion generated during locomotion using arboreal vs. terrestrial substrates (Barnett & Napier, 1953; Carleton, 1941; DeSilva, 2009; Latimer *et al.*, 1987; Stern & Susman, 1983).

Despite recent studies showing the importance of studying fibular diaphyseal strength to further our understanding of the degree of arborealism of early hominins (Marchi *et al.*, 2019), more is needed about the morphology of proximal and distal ends. Concerning the distal end, previous studies performed using traditional morphometrics showed that great apes possess a more anteriorly oriented lateral malleolus and more downward facing fibulotalar articular facets as well as a more craniocaudally elongated subcutaneous triangular surface (STS) than humans (Marchi, 2015b; Stern & Susman, 1983). A recent 3D geometric morphometric (3D-GM) study revealed that the distal fibular morphology indicates arboreal vs. terrestrial locomotor

patterns within extant hominids (Marchi *et al.*, 2022). The study confirms and expands previous morphological observations regarding the orientation of the lateral malleolus, fibulotalar articular facets, and STS proportions, implying a wider peroneal groove and a deeper and broader malleolar fossa in great apes than in humans. On the other hand, the proximal fibular epiphysis is rarely investigated in humans except for clinical purposes, and even less data is available for great apes.

1.1 Anatomy and biomechanics of the proximal fibula and proximal tibiofibular joint in modern humans

The proximal fibula (Figure 1, Figure S1) in modern humans is composed of the fibular head, a mediolaterally expanded bone protrusion surmounting the fibular neck and accommodating the proximal tibiofibular articular surface (Martini, Timmons & Tallitsch, 2009). The proximal extremity of the fibular head bears the styloid process, serving as the insertion for the popliteofibular ligament (PFL). At the same time, a lateral bulge holds the insertion area for the *m. biceps femoris* (knee flexor) tendon (BT) and the fibular collateral ligament (FCL) (Song *et al.*, 2018; 2020; Takahashi *et al.*, 2017).

The anatomy of the proximal tibiofibular joint (PTFJ) is a plane synovial joint between the articular facet on the lateral condyle of the tibia and the facet on the head of the fibula (Sarma *et al.*, 2015). An articular capsule covers the joint, attaching just beyond the articular surfaces of the tibia and the fibula. This capsule is further reinforced by the thick anterior (APTFL) and thinner posterior (PPTFL) proximal tibiofibular ligaments, which also insert through a variable number of bundles into the fibular head anterolaterally and posteriorly (Anavian *et al.*, 2018; Scarciolla *et al.*, 2021). The APTFL inserts into an anterior protrusion in which the anterior border culminates, separating the medial from the lateral surface (White, Black & Folkens, 2011). Immediately lateral to this anterior protrusion and below the attachment of BT and FCL, the proximal origin to *m. peroneus longus* (PL), which acts to evert and plantarflex the ankle) is found (Sammarco & Mangone, 2000). Posteriorly, the PPTFL also inserts into a posterior protrusion that superiorly delineates the horse-shoe-shaped attachment area of the *m. soleus*. The anteromedial surface of the fibular head

hosts the proximal insertion of the m. *extensor digitorum longus* (EDL) (White *et al.*, 2011).

Several studies have classified the PTFJ based on the shape and orientation of the joint surface, and significant variability has been reported (Barnett & Napier, 1952; Eichenblat & Nathan, 1983; Martin & Saller, 1959; Ogden, 1974a; 1974b). In general, the PTFJ ranges from a horizontal, planar articular surface, considered more mobile (i.e., in terms of the fibula), to more oblique articular surfaces, at varying angles with the longitudinal axis of the fibula and with different outlines and depths, which are considered less mobile.

The PTFJ is primarily involved in the dissipation of torsional stresses applied at the ankle joint (Ogden, 1974a; 1974b) while also contributing to the dissipation of lateral tibial bending moments and tensile forces, rather than compressive weight bearing (Ogden, 1974a; 1974b). Indeed, Evans and Bang (1966) found that proximal fibulae were stronger (ultimate tensile and single shearing strength), stiffer (modulus of elasticity), and had a greater tensile strain (% elongation) in comparisons to femurs (see also Preuschoft, 1970). The fibula contributes to the dissipation of tensile forces via the interosseous membrane and at the PTFJ, which rotates externally when the ankle is in dorsiflexed position (Andersen, 1985; Bozkurt *et al.*, 2003).

Lambert (1971), on the other hand, suggested that the fibula has an additional weight-bearing function, with approximately one-sixth of the static load applied at the ankle transmitted to the PTFJ, in agreement with other studies that found different degrees of fibular load sharing (8-19%) at varying ankle eversion/inversion angles (Funk *et al.*, 2007). In addition, Lambert (1971) suggested that the force transmitted to the PTFJ was generated by the fibulo-talar articulation and facilitated by the inferior tibiofibular ligaments and the interosseous membrane. Similarly, under axial loading, the lateral malleolus migrates distally and laterally to the tibia (Wang *et al.*, 1996).

The PTFJ is also involved in the anteroposterior shifting of approximately 1 cm in both directions according to flexion/extension movements at the knee, moving anteriorly when the knee is in a flexed position and posteriorly when the knee is extended (Ogden, 1974a; 1974b).

1.2. The proximal fibula in human and nonhuman great apes

The fibular head of great apes does not possess a styloid process; therefore, the fibula articulates directly below the tibial lateral condyle. The appearance of the PTFJ surface is well-defined in great apes and contributes to a superiorly flat fibular head (Martin & Saller, 1959). On the other hand, the human PTFJ articulates against the lateral side of the lateral tibial condyle and has a greatly variable shape (Barnett & Napier, 1952; Eichenblat & Nathan, 1983; Odgen, 1974a; 1974b). The fibular neck is more anteroposteriorly slender in humans than the rest of the fibular shaft. In contrast, in great apes, the robusticity of the neck is similar to the rest of the shaft (Susman & Stern, 1982). In humans and African apes, the FCL has a cord-like form, stabilizes the knee laterally, and inserts distally to the anterolateral protrusion of the fibular head (Aiello & Dean, 1990). This anterolateral protrusion is also the attachment area of the distal insertion of the BT. This insertion site is shared among all hominoids, where in African apes, the BT also attaches distally onto the lateral condyle and tuberosity of the tibia, and in Asian apes onto the capsule of the knee joint and variably onto the FCL and the lateral epicondyle of the femur. The two heads of the *m. biceps femoris* are fused at their distal insertion in chimpanzees and modern humans but not in *Pongo* and *Gorilla* (Ferrero *et al.*, 2012). Kaseda and colleagues (2008) describe the unique morphology of the femoral part of the *m. biceps femoris* in orangutans and suggests its adaptation to a more arboreal behavior due to similarities with Old World monkeys.

In humans, the characteristic shape of the fibular head is determined by the prominence of the styloid process, which culminate both the anterolateral and posterior protuberances and offer attachment to the BT and the PPTFL, respectively (Song *et al.*, 2018; 2020). The apex of the styloid process in humans consists of the attachment site for the PFL, one of the critical structures forming the popliteus complex (LaPrade & Bollom, 2001; Zeng *et al.*, 2011). The primary function of the PFL is to resist the posterior dislocation of the lateral condyle of the tibia (Pasque *et al.*, 2003). Kaplan (1957) reports that the connection between the fibular head and the popliteus complex through the PFL is also found in chimpanzees. However, this configuration is rarely reported, probably due to a lack of precise terminology in evaluating the popliteus complex, which is often described without distinguishing the fibular

component of its tendon (PFL). In all hominoids, the m. *popliteus* originates from the lateral meniscus. It is reported that in orangutans, this muscle may have two heads as its origin, one of which is located on the fibular head (Ferrero *et al.*, 2012). In modern humans, the muscle flexes and laterally rotates the femur with a fixed tibia or medially rotates the tibia with the fixed femur, "unlocking" the fully extended knee as flexion begins (Aiello & Dean, 1990). In apes, the muscle is a lateral stabilizer of the knee joint (Aiello & Dean, 1990).

Immediately distal to the attachment of BT and FCL, the anterolateral aspect of the fibular head hosts the proximal insertion of the PL in all hominoids, which extends to the lateral aspect of the proximal fibula shaft in all taxa but *Pan* (Ferrero *et al.*, 2012). In humans, the PL, together with the m. *peroneus brevis*, is active during the second half of the stance phase when the weight is transferred to the anteromedial portion of the foot (Aiello & Dean, 1990; Reeser *et al.*, 1983; Stern & Susman, 1983). In addition, Bavdek and colleagues (2018) showed that the electromyographic activities of peroneal muscles increased when humans walked on a medially inclined ramp, therefore everting the inverted foot (Marchi *et al.*, 2022). Peroneal muscles are recruited in chimpanzees mainly during the support phase of locomotion on either vertical or horizontal trunks when everting the foot (Stern & Susman, 1983) and regulate the transfer of weight on the medial part of the inverted foot during climbing in great apes (Marchi *et al.*, 2022).

In all hominoids, the m. *soleus* inserts proximally on the posterior aspect of the head of the fibula, immediately distal to the posterior projection that serves as the attachment site for the PPTFL. In modern humans, the proximal insertion site is horseshoe-shaped, and a second proximal insertion site forms a bony ridge on the medial surface of the tibia. In great apes, on the other hand, the proximal insertion of the m. *soleus* is usually confined to the fibular head and occasionally can have a proximal accessory insertion on the tibia (Aiello & Dean, 1990; Prejzner-Morawska & Urbanowicz, 1981). The elongation of the m. *soleus* insertion shape was previously suggested as determining the fibular head shape in humans, which culminates in the styloid process, versus that in apes, which possess a flat head (Martin & Saller, 1959). The m. *soleus*

of *Gorilla* and *Pan* is bulkier than that of the Asian apes (Ferrero *et al.*, 2012). In humans, the whole m. *triceps surae* is twice as heavy as in *Pan*, reflecting its involvement in lifting the whole-body weight in bipedal locomotion (Aiello & Dean, 1990). A medial expansion of m. *soleus* seen in apes may be functionally related to an arboreal lifestyle with significant amounts of vertical climbing (Hanna & Schmitt, 2011), with medial fibers more active than lateral fibers in foot inversion (O’Connell, 1958).

The anteromedial surface of the fibular head provides attachment for the m. *extensor digitorum longus*, an extensor of the II-IV toes (Aiello & Dean, 1990), in all hominoids. In *Gorilla*, there is also an origin from the anterior surface of the shaft of the fibula (Ferrero *et al.*, 2012).

1.3. Aim of the work

In this study, we provide a detailed comparative assessment of shape variation of the proximal fibula in extant humans and great apes using 3D-GM to detail the variation of proximal fibular shape in each taxon and investigate a possible link with locomotor and positional behaviors. The 3D-GM approach has been applied to human and nonhuman primate bones by many recent studies to address functional questions providing valuable information for the assessment of shape variation of postcranial skeletal elements and functional interpretation of fossil material (Almecija *et al.*, 2013; Frelat *et al.*, 2017; Harmon, 2006, 2007, 2009; Harcourt-Smith *et al.*, 2008; Marchi *et al.*, 2022; Sorrentino *et al.*, 2020a, 2020b; Turley, Guthrie & Frost, 2011).

Based on previous studies outlined above, we expect that the shape of the fibula in extant hominids will significantly differ between humans and great apes because the different locomotor and postural repertoires entail different loading regimens applied at the knee in flexion (Aiello & Dean, 1990; Isler, 2005; Scott *et al.*, 2007) and at the ankle in dorsiflexion and eversion (Ogden, 1974a, 1974b).

In particular, we expect to find the following:

(1) a more horizontal proximal tibiofibular articular surface in great apes than in humans, previously linked to higher mobility of the knee (Frelat *et al.*, 2017; Sylvester & Pfisterer, 2012; Sylvester, 2013; Tardieu, 1999) and ankle (Barnett & Napier, 1953; DeSilva, 2009; Latimer *et al.*, 1987; Stern & Susman, 1983);

(2) a superiorly elongated fibular head (i.e., presence of the styloid process) in humans in comparison to a flat fibular head in great apes, contributing to the knee ‘unlocking mechanism’ in bipedal standing in the former group (Aiello & Dean, 1990);

(3) an anterolateral expansion of the fibular head suggesting more powerful hamstrings and peroneal muscles in great apes than in humans, linked to the stabilization of the inverted foot during vertical climbing and walking/clambering (Stern & Susman, 1983);

(4) a proximo-distally elongated insertion of the m. *soleus* in humans due to its greater mass relative to the whole m. *triceps surae*, with a medially expanded insertion area in apes, as it is considered an indicator of a greater degree of arborealism (Hanna & Schmitt, 2011).

Finally, a further goal of this study is to lay the ground for further investigations of the proximal fibula of extinct hominins, as this element is preserved in the fossil record (e.g., *Australopithecus sediba*, DeSilva *et al.*, 2013; StW 573 “Little Foot”, Heaton *et al.*, 2019; *Homo floresiensis*, Jungers *et al.*, 2009; *Homo neanderthalensis*, Trinkaus, 1983) but rarely quantitatively compared or analysed.

2. Materials and methods

2.1. The sample

The analyzed sample includes a total of 94 fibulae belonging to 37 *H. sapiens*, 15 *Gorilla* (*Gorilla gorilla* = 12; *Gorilla beringei* = 3), 17 *Pongo* (*Pongo abelii* = 8; *Pongo pygmaeus* = 9), and 25 *Pan* (*Pan troglodytes* = 24; *Pan paniscus* = 1) (Table 1). All fibulae belong to adult individuals with fully fused epiphyseal lines according to the visual estimation criteria (Belcastro *et al.*, 2019) and without signs of pathology in the skeleton. The fibulae were selected based on their general good state of preservation. For each individual, the left fibula was used. When the left fibula was unavailable, the right one was digitally mirrored.

Three-dimensional surface meshes of the fibula belonging to the University of Bologna (UNIBO) institution were digitized through computed tomography (CT), utilizing a Revolution Discovery CT dual energy at Istituto Ortopedico Rizzoli (Bologna, Italy) (slice thickness and increment at 0.625 mm, voltage 100

kV, X-ray tube current 360 mA, reconstruction at 40 keV with “Detail” filter, voxel size ranging from 0.39 to 0.507 x 0.625 mm). The reconstructed DICOM (16-bit gray-scale, signed) images were then processed with Avizo 9.2 (Thermo Fisher Scientific) for image segmentation with a modified version of the half-maximum height (HMH) protocol (Spoor *et al.*, 1993; Coleman & Colbert, 2007). Lastly, an isosurface was generated for each segmentation.

Three-dimensional surface meshes of ape fibulae belonging to the State Zoological Collection in Munich (SZCM), Germany, and the University of Zurich (UZH), Switzerland, were obtained using medical CT scanning. The SCM specimens were collected at the Munich Institute for Radiology Ludwig Maximilian University (Munich, Germany) on a GE Discovery CT750 HD medical CT scanner (slice thickness 0.625 mm, slice increment 0.3 mm, voltage 120 kV, X-ray tube current 99 mA, reconstructing algorithm bone, pixel size 460 μ m). The UZH specimens were collected at the University Hospital of Zurich (Zurich, Switzerland) on a Siemens Somatom Definition Flash (slice thickness 0.6 mm, slice increment 0.3 mm, voltage 120 kV, current 19 mA, reconstructing algorithm bone, pixel size 600 μ m). Similar to the UNIBO sample, the SZCM and UZH samples were segmented utilizing the HMH segmentation protocol detailed previously. All UNIBO, UZH, and SZCM samples consisted of dry bone specimens.

Selected fibulae belonging to the Division of Mammals, National Museum of Natural History, Smithsonian Institution, and the Mammal Collections at the American Museum of Natural History were obtained via MorphoSource (<https://www.morphosource.org/>). The specimens include dry bone samples, and the digital models were obtained either by laser scanning or CT scanning. MorphoSource Media IDs and Institution collection numbers of these specimens are displayed in the S1 Appendix.

Other ape specimens used in this study were obtained via Digital Morphology (KUPRI) from the Primate Research Institute’s (PRI) collection of CT scans. For these specimens, scan resolution (X, Y) ranged from 0.133-0.761 mm, with a slice thickness (Z) of 1-2 mm. The specimens included dry bones, fresh cadavers, and other samples with frozen or immersed soft tissue.

2.2. 3D Geometric Morphometric analysis

A 3D template configuration (Figure 1, Table 2) of 16 fixed landmarks, 25 curve semilandmarks, and 101 surface semilandmarks captured the proximal extremity of the fibula. The template was created in Viewbox 4 to cover major muscle, ligament, and tendon attachment sites and articular surfaces on the proximal fibula (Figure 1, Table 2). Next, Viewbox software was utilized to apply the template configuration to the sample of proximal fibulae (targets), with semilandmarks sliding on curves and surfaces to minimize thin-plate spline (TPS) bending energy between the targets and the template (Slice, 2006). Following that, semilandmarks can be considered geometrically homologous among specimens (Gunz, Mitteroecker & Bookstein, 2006; Gunz & Mitteroecker, 2013). The template repeatability (i.e., high intra-observer agreement) and reproducibility with different scanning devices were tested in a previous study (Pietrobelli *et al.*, 2022). Landmark and semilandmark raw coordinates used are available in the S2-S3 Appendix. After importing raw coordinates in R (version 4.0.3) (R Core Team, 2020), a further sliding step was performed against recursive updates of the Procrustes consensus (R package “geomorph”; Adams *et al.*, 2018), while a Procrustes superimposition was computed. This procedure allowed the conversion of raw coordinates into standardized, scaled, centered, and oriented shape coordinates (i.e., Procrustes coordinates) via Generalized Procrustes Analysis (GPA) (Slice, 2006; Rohlf & Slice, 1990). Outliers from Procrustes consensus were detected utilizing the function `plotOutliers()` provided in this package. The centroid size (CS) was also calculated and used as a proxy for the size of the proximal end of the fibula (Slice, 2006).

Procrustes coordinates were then subjected to a principal component analysis (PCA) to explore shape variations among the different genera (Schlager, 2017). Finally, principal component (PC) scores were evaluated and compared among genera using a one-way ANOVA, with subsequent *posthoc* pairwise comparisons, and visualized as violin plots.

Visualization of shape changes along the principal axes and means based on genera were obtained by TPS deformation (Bookstein, 1991) of the Procrustes grand mean shape surface utilizing the R package “Morpho” v. 2.8 (Schlager, 2017). In addition, visualizations of the mean shape distances among mean

configuration according to genera and Procrustes consensus were computed utilizing the function `localmeshdiff()` from the package “Arothron” v. 2.0.3 (Melchionna *et al.*, 2020; Profico *et al.*, 2021).

Procrustes ANOVA was used to test shape differences among genera, utilizing Procrustes distances among specimens and using a residual randomization procedure (RRPP = T, iterations = 1,000), with the R package “geomorph” v. 3.3.2 (Adams *et al.*, 2018). Differences in size among genera were evaluated using ANOVA and subsequent *posthoc* tests and visualized in relative box plots. Procrustes distance distribution was also visualized by computing density values of Procrustes distances of each specimen to the Procrustes consensus, separated by genus.

The form-space (i.e., shape plus size) PCA was computed by augmenting the Procrustes shape coordinates of each dataset of Procrustes coordinates by the natural logarithm of CS (lnCS) (Klingenberg, 2016). Linear correlation among CS and the PC scores along the first two shape PCs was assessed by a Pearson's correlation test to assess whether the distribution of individuals in the shape space PCA plot was influenced by size. All these analyses were then repeated, excluding the *H. sapiens* sample and considering the great apes' sample only.

3. Results

A summary of the morphological differences observed among the sampled genera is presented in Table 3 and detailed in Figure 2.

3.1. All extant hominoid genera

Figure 3 shows the shape space PCA scatterplots of *Pan*, *Gorilla*, *Pongo*, and *H. sapiens* and extreme shape variations along the first three PCs, which account for 63.88 % of the total variance (PC1: 47.47%; PC2: 9.30%; PC3: 7.11%).

The plot of PC1 against PC2 (Figure 3) clearly shows the separation along PC1 between *H. sapiens*, plotting towards PC1 positive scores, and great apes, plotting towards PC1 negative scores (Figures 3 and 4). While all great apes taxa show some degree of overlap along PC1, *Pongo* and *Pan* PC scores distribution differs significantly (Table 4). PC1 positive extreme shape, characterizing *H. sapiens*, is driven mainly by a well-marked styloid process, an anteroinferior, posterior superiorly angled PTFJ, and presents a squared head

in lateral view, a thicker neck, a flat medial surface, a raised anterior border, a moderately anteriorly facing and well-defined flat area of insertion for PL/BT, and an inferiorly elongated attachment area of the m. *soleus*. PC1 negative extreme shape, characterizing great apes, is mainly driven by a horizontal PTFJ with an absent styloid process, a rectangular head in lateral view, a slender neck, a concave medial surface, an inferiorly elongated PL/BT insertion area which is less expanded laterally in comparison to all other extremes, and a posteromedially expanded m. *soleus* attachment area that is less defined and surmounted by a rounded protrusion posteriorly.

Along PC2, *H. sapiens* overlaps with all great apes. *Pan* clusters with more positive scores within great apes and is significantly different from the other genera (Table 4). *Gorilla* and *Pongo* overlap extensively, with *Pongo* showing greater dispersion. *Pan* shows a rounded head in lateral view, a slender neck, an approximately horizontal PTFJ, a slightly concave medial surface, an inferiorly elongated PL/BT insertion area, and a posteromedially expanded m. *soleus* attachment area that is less defined and surmounted by a rounded protrusion posteriorly. *Pongo* and *Gorilla* show, in comparison, a more rounded fibular head and a more pronounced laterally expanded PL/BT insertion.

All taxa overlap extensively along PC3, with no significant differences ($p > 0.05$) in distribution (Figures 3 and 4). However, all groups diverge from one another regarding Procrustes distances, except for gorillas and orangutans (Table 5, Figure S2).

Mean shapes for each genus are represented in Figure S3 and display a trend already observed with the extreme shapes that distinguish humans from great apes.

Regarding the CS comparisons, *Gorilla* and *Pongo* show the most prominent and undersized proximal ends, respectively (Figures 4), and are significantly different from all other genera. *Homo sapiens* and *Pan* display comparable CS means and distributions. All PC scores along the three first shape PCs do not significantly correlate with CS. A clear separation between *H. sapiens* and great apes is present in the form space PCA along PC2, and *Pongo* is clearly separated from *Gorilla* along PC1 with *Pan* plotting in between (Figure 5).

3.2. Apes only

Figure 6 shows the shape space PCA scatterplots for *Pan*, *Gorilla*, and *Pongo* and extreme shape variations along the first three PCs, which account for 49.59% of the total variance (PC1: 23.92%; PC2: 13.60%; PC3: 12.07%). Again, more apparent separation is observed among the great apes when humans are excluded from the analysis.

The plot of PC1 against PC2 (Figure 6) shows that along PC1, *Pan* plots towards negative scores, *Pongo* towards positive scores, and *Gorillas* is in between, slightly overlapping with both *Pan* and *Pongo* towards positive scores. Along PC1, all great apes differ significantly from one another (Figure 7 and Table 6). *Pongo* (PC1 positive) shows a slightly circular fibular head in lateral view, a narrower neck, with an anterosuperior posterior-inferiorly angled PTFJ oblique PTFJ, a concave medial surface right distal the PTFJ border, a defined anterior border, a marked laterally protruding insertion areas of PL/BT, a narrow, deep attachment for the FCL, and a less inferiorly elongated, sub-circular m. *soleus* attachment area, superiorly delineated by a well-rounded protrusion for the PPTFL.

Pan (PC1 negative) displays a rectangular fibular head in lateral view and a slender neck, a horizontal PTFJ, a flat medial surface, and a well-delineated area for PL/BT, which is inferiorly expanded but less laterally projected, with a superior impression for FCL and a deep, oval attachment of m. *soleus*, with posteromedial expansion.

All taxa overlap extensively along PC2 or PC3 (Figures 6 and 7). The only significant difference is between *Pongo* and *Gorilla* along PC2 (Table 6). All groups differ in the Procrustes distance (Table 7, Figure S4). Shape variation along PC2 is similar to that observed along PC1. It primarily refers to a more lateral projection of PL/BT (PC2 positive) and a slightly anteroposteriorly oblique PTFJ towards positive scores, with a posteromedial expansion for the m. *soleus* attachment, less inferiorly elongated in *Pongo* (PC2 negative). PC3 mainly refers to an inferiorly elongated and narrow (PC3 positive) compared with an anteroposteriorly expanded and shorter (PC3 negative scores) fibular head.

Mean shapes for each genus of the great apes display similar morphologies to

those already observed in extreme shape reconstructions along PCs (Figure S5).

Concerning CS comparisons and form space variations, *Gorilla* and *Pongo* show the most significant and minor proximal epiphyseal size, respectively (Figures 7 and 8). The form space bivariate plot of PC1 against PC2 shows segregation among the three genera. None of the PC scores along the three first PCs significantly correlates to CS.

4. Discussion

In this study, we have provided a thorough morphometric description of the proximal fibula in extant hominids by the 3D-GM approach. In addition, we hypothesized significantly different shape changes among extant significant ape taxa and humans in response to these groups' different ankle and knee loading regimens. Our results are consistent with our hypotheses and highlight variation comparable to previous evaluations of distal fibular morphology among extant hominids (Marchi, 2015b; Marchi *et al.*, 2022; Stern & Susman, 1983), further highlighting the functional role of the fibula and its potential in inferring mobility patterns in early hominins.

In particular, in humans, we expected to find a more angled proximal tibiofibular joint (PTFJ), the presence of the styloid process, reduced anterolateral and inferior expansion of the hamstrings and peroneal muscles insertion areas on the fibular head as well as a deep insertion of the m. *soleus*. In contrast, great apes were expected to possess a more horizontal PTFJ, a superiorly flat fibular head, an anterolateral and inferior expansion of the hamstring and peroneal muscles insertion areas onto the fibular head, and a defined but shallower, more medially expanded m. *soleus* insertion. The presence of these shape changes in our sample is in agreement with the expected higher mobility of the ankle (Barnett & Napier, 1953; DeSilva, 2009; Latimer *et al.*, 1987; Stern & Susman, 1983) and knee joints (Frelat *et al.*, 2017; Sylvester, 2013; Sylvester & Pfisterer, 2012; Tardieu, 1999) in great apes and with the unique knee 'unlocking mechanism' present in modern humans when initiating knee flexion (Aiello & Dean, 1990). Moreover, the observed anterolateral, inferior and medial expansion of the hamstring and peroneal tendon insertions in great apes agrees with the need for stabilization of an

inverted foot during vertical climbing and walking/clambering (Stern & Susman, 1983) and with a greater degree of arborealism (Hanna & Schmitt, 2011). On the other hand, the observed deeper insertion of the m. *soleus* in modern humans may indicate its relatively greater muscle mass than the whole *triceps surae*, necessary for bipedal walking and running (Hanna & Schmitt, 2011).

4.1. Differences between humans and great apes

The results of our morphometric comparison between the proximal fibula of modern humans and extant great apes provide further support to the functional role of the fibula in humans, highlighting a suite of morphological features that appear correlated with bipedalism (Harcour-Smith, 2015). For example, the more angled PTFJ (Figure 2, Figure 3) in humans may indicate a less mobile articulation (Eichenblat & Nathan, 1983; Odgen, 1974a; 1974b; Sarma *et al.*, 2015). Indeed, in the past, the angle of the PTFJ was associated with a reduced dorsiflexion axis inclination at the ankle (Barnett & Napier, 1953; Odgen, 1974a; 1974b), consistent with the requirement for bipedal locomotion (Susman & Stern, 1983), but never quantified. In humans, the degree of inclination of the PTFJ surface is remarkably variable, ranging from 5° to 80° (Eichenblat & Nathan, 1983). Ogden (1974a; 1974b) found that oblique joints (>20°) have smaller articular surface areas decreasing mobility and reducing the joint ability to accommodate torsional forces, with a higher incidence (70%) of subluxation or dislocation. In contrast, in humans, the less inclined PTFJ enables greater mobility and is, therefore, less prone to injury (Alves-da-Silva *et al.*, 2019). However, horizontal PTFJ in humans is also associated with a higher risk of osteoarthritis of the knee's medial tibiofemoral compartment. The lateral tibial plateau is more supported by a horizontal PTFJ resulting in a non-uniform settlement of the whole tibial plateau, ultimately accentuating *varus* knee as well as further knee malalignment and provoking osteoarthritis in the medial compartment (Chang *et al.*, 2020; Huang *et al.*, 2019).

Both these mechanisms may be related to hindlimb adaptations for obligate bipedalism in humans: the angled PTFJ observed in humans (Eichenblat & Nathan, 1983) may reflect the reduced torsional forces to which the fibula is

subjected during ankle dorsiflexion in bipedality. Ankle dorsiflexion produces a posterolateral shift of the fibular head (Barnett & Napier, 1953; Odgen, 1974a; 1974b) which is, therefore, more accentuated in nonhuman apes than in humans (Susman, 1983; De Silva, 2009). The decreased mobility of the fibula that an oblique PTFJ concurs to limiting ankle mobility, but at the same time, might cause the proximal fibula to be more prone to dislocation. A predominantly horizontal PTFJ in humans (< 5%, Eichenblat & Nathan, 1983) would theoretically provide greater knee lateral stabilization in flexion and extension but would ultimately lead to joint malalignment (*varus* knee). The accentuated *varus* knee in humans contrasts the need to minimize transverse shear stresses achieved with a *valgus* knee (Tardieu & Trinkaus, 1994). The latter may be linked to the different orientations of ground reaction forces in bipedal walking in humans (more anteroposteriorly oriented) and chimpanzees (laterally oriented) (Johnson, O'Neill & Umberger, 2022), also in agreement with the relative fibular gracility seen in humans compared to great apes (Marchi, 2007).

Our 3D-GM analysis reveals that the fibular head of humans has symmetrical anteroposterior and mediolateral proportions, with well-rounded but diminutive anterior and posterior projections, which anchor the respective anterior and posterior tibiofibular collateral ligaments, and are located more superiorly than in great apes (Figure 2, Figure 3, Figure S3). This superior, rounded configuration may be implicated in the ligament lesion, and PTFJ dislocation mechanism Ogden (1974a; 1974c) described as the result of a sudden inversion and plantar flexion of the foot in bipedal locomotion, with simultaneous flexion of the knee and concomitant twisting of the limb, as also verified by 3D displacements analysis (Alves-da-Silva *et al.*, 2019). Moreover, it was revealed that full extension of the knee fully recruits the APTFL (Alves-da-Silva *et al.*, 2019), suggesting its significance for human bipedal walking on an extended hindlimb. This reduces the energy cost of locomotion compared with chimpanzees walking both quadrupedally and bipedally (Pontzer *et al.*, 2009; Sockol *et al.*, 2007).

The human fibular head is also characteristically surmounted by a styloid process (Figure 2, Figure 3, Figure S3). This feature may play a crucial role in

supporting the lateral compartment of the knee when in full extension (Tardieu *et al.*, 1999) since it provides attachment for the PFL to the popliteus tendon. In addition, these soft structures stabilize the whole posterolateral tendon and ligaments complex, the posterolateral corner of the knee by preventing posterior translation, *varus* angulation, and primary external rotation of the tibia relative to the femur (LaPrade *et al.*, 2001; 2003).

The morphology of the muscle attachment areas on the fibular head in humans may also reflect functional adaptations to bipedal walking. In humans, the long head of the *m. biceps femoris* inserts superiorly onto the rounded, anterolateral portion of the fibular head and expands posteriorly onto the styloid process through its interfacing components (direct arms of short and long heads, an anterior arm of long head) (LaPrade *et al.*, 2001; 2003) (Figure 2, Figure 3, Figure S3). The role of this muscle in obligate bipedal locomotion is crucial due to its configuration within the hamstring. In humans, as the lever for the hamstrings is lengthened, the muscle moment arms (at the hip and/or knee) are shortened, decreasing the power-generating capacity of the muscles but favoring speed generation in the form of angular velocity, essential for running (Hogervorst & Vereecke, 2015; Kozma *et al.*, 2018). It has been observed that humans possess shorter *m. biceps femoris* moment arms at the knee relative to femur length than great apes (Payne *et al.*, 2006b; Visser *et al.*, 1990), in agreement with the less posteroinferiorly elongated distal insertion onto the fibular head (Figure 2, Figure 3, Figure S3).

Concerning the fibular proximal insertion of the *m. peroneus longus*, our 3D-GM analysis shows that humans possess a relatively flat, narrow, anteriorly facing attachment, in contrast to an anterolaterally expanded concave attachment observed in great apes. In humans, peroneal muscles are active during the support phase of locomotion, similar to great apes (Jungers, Meldrum & Stern, 1993; Stern & Susman, 1983) (Figure 2, Figure 3, Figure S3). EMG studies have shown that in humans, peroneal muscles are more active when everting the inverted foot (Bavdek *et al.*, 2018), suggesting their essential role in laterally stabilizing the inverted ankle joint while traversing a medially inclined ramp. Peroneal muscles in humans also have been reported to form concave furrows along the anterior fibular surface of the diaphysis in

correlation with a combined effect of age and mechanical loading (Chevalier & Tignères, 2020; Hagihara & Nara, 2016). While no specific studies on peroneal anatomy and electromyography were performed on great apes, previous research on human peroneal muscles activation and fibular diaphyseal anatomy (Bavdek *et al.*, 2018; Chevalier & Tignères, 2020) allow us to suggest that the lack of a concavity in the anterior surface of the fibula is indicative of lower peroneal activity in humans than in great apes. This finding agrees with previous studies that suggested a connection between a wider peroneal groove and fossa, observed at the distal fibular extremity, and larger, powerful peroneal muscles (Marchi *et al.*, 2015b; Marchi *et al.*, 2022).

The present study also showed a proximal insertion of the m. *soleus* on the fibula that is narrower, deeper, and more posteriorly elongated in humans than in great apes (Figure 2, Figure 3, Figure S3). This finding is consistent with the m. *soleus* contributing to over 60% of m. *triceps surae* mass in humans (Hanna & Schmitt, 2011) and generating the central part of work/energy at the ankle joint throughout the entire stance phase of bipedal running (Bohm *et al.*, 2021; Lai *et al.*, 2015). The m. *triceps surae* is twice as heavy relative to human body size than in chimpanzees (Aiello & Dean, 1990; Zihlman & Brunner, 1979). It is also responsible for supporting the body weight after touchdown throughout the walking stance phase and for lifting the body's entire weight in the second half of the stance phase (Hanna & Schmitt, 2011).

4.2. Differences among great apes

In great apes, the observed horizontal PTFJ agrees with a highly mobile articulation necessary to accommodate the high degree of ankle dorsiflexion and eversion-inversion (Ogden, 1974a; 1974b) that characterize, for example, vertical climbing (DeSilva, 2009; Holowka *et al.*, 2017) (Figure 2, Figure 6, Figure S5). The more horizontal PTFJ in great apes may also facilitate anteroposterior excursion of the proximal fibula proximally, as observed for humans (Scott *et al.*, 2007). Previous studies on the kinematics of the knee joint of great apes during bipedal or quadrupedal terrestrial walking (Finestone *et al.*, 2018; O'Neill *et al.*, 2015) and climbing (DeSilva, 2009; Isler, 2005) did not include the PTFJ in their evaluations. However, these studies noted a consistently wider range of motion and distinctive patterns of knee and ankle

flexion in great apes than in humans and specific trends within great apes. Indeed, our 3D-GM analysis results show that all great apes have a more horizontal PTFJ than humans in mean shape (Figure 2, Figure 3, Figure 6, Figure S3, Figure S5). However, differences among the great apes are present. In particular, chimpanzees display a markedly horizontal PTFJ, coherent with a higher flexibility of the knee and ankle during vertical climbing (Barnett & Napier, 1952; Crompton *et al.*, 2010; Ogden, 1974a, 1974b), while orangutans and gorillas show a more posteriorly tilted articulation. This feature in orangutans could be linked to PTFJ stabilization during knee extension while walking bipedally in an arboreal setting (Thorpe *et al.*, 2007), similar to that observed for tilted PTFJ in humans (Eichenblat & Nathan, 1983; Ogden, 1974a; 1974b). In gorillas, kinematic data are needed to understand how this pattern may relate to specific variations of fibular excursions associated with knee and ankle movements. However, in humans, a larger anterior displacement on the fibular head is observed with knee varus load in flexion (Scott *et al.*, 2007). Therefore, we tentatively suggest that the orientation of the PTFJ in orangutans and gorillas may be similarly preventing anterior displacement of the fibula and be linked to their wider bicondylar angles than in chimpanzees as knee flexion increases (Lovejoy, 2007), which also mitigates lateral displacement and varus loading of the knee. This shape is consistent with a broader range of motion of thigh abduction (Isler, 2005), which may result in a mediolateral bending load increase on the fibula, acting as a lateral stabilizer, as observed in humans (Wang *et al.*, 1996). A similar explanation may also account for the differences observed for the *m. biceps femoris* and proximal FCL insertion on the fibular head of orangutans, which are more laterally protruding and deeper than in African great apes (Figure 2, Figure 6, Figure S5). The fibular head is pulled posteriorly as the *m. biceps femoris* and FCL becomes taut (Semonianan *et al.*, 1995). Indeed, in humans, when the knee is semi-flexed, the *m. biceps femoris* contributes to thigh abduction and lateral rotation (LaPrade *et al.*, 2003). Suppose a similar function is assumed for other hominids (Kumakura, 1989), a lateral bony protrusion for the *m. biceps femoris* in orangutans may be mechanically advantageous for knee extension/flexion while the thigh is laterally rotated (Isler, 2005). On the other hand, African

great apes lack this feature, which may be related to the modest activity of this muscle during knuckle-walking (Kumakura, 1989).

Our results show that all great apes separate from one another (Figure 6) due to distinctive proximal fibular morphology. Previous studies found significant differences in relative (to the tibia) fibular strength (Marchi, 2007; 2015a) and distal fibular morphology (Marchi *et al.*, 2022) among taxa that engage more frequently in vertical climbing behavior (*Pan* and *Pongo*) compared to those that are more terrestrial (*Homo* and *Gorilla*). The authors linked these differences to larger peroneal muscles in the former, a feature that has generally been associated with arboreal great apes (Aiello & Dean, 1990; McLean & Marzke, 1994; Stern & Susman, 1983). These results are supported by our study, in which orangutans show a concave and markedly anterolaterally protruding attachment area for the peroneal muscles, with an expansion best seen in the medial view. In contrast, gorillas and chimpanzees display a more anterior insertion area (Figure 2, Figure 6, Figure S5). As detailed above, m. *peroneus longus* is recruited for the lateral stabilization of the inverted foot (Bavdek *et al.*, 2018) and orangutans perform extreme inversion and eversion of the foot when traversing trees (Manduell *et al.*, 2012; Oishi *et al.*, 2012; Thorpe *et al.*, 2007) and when supporting themselves on horizontal branches or vertical tree trunks (Thorpe & Crompton, 2006). The anterolateral protrusion attachment of m. *peroneus longus* of orangutans may facilitate these sagittal movements by increasing the bony leverage in the lateral plane. However, to our knowledge, no empirical demonstration of this has been provided for great apes. In addition, in gorillas, this insertion also shows a mild lateral expansion that is not seen in chimpanzees. EMG studies in humans showed that this muscle's anterior neuromuscular compartment (NMC) contributed to both eversion and plantarflexion movements, whereas the posterior compartment mainly contributed to eversion only (Bakkum *et al.*, 1996; Mendez-Rebolledo *et al.*, 2021). As gorillas engage in terrestrial plantigrade quadrupedalism with propulsive plantarflexion (Straus, 1940), their anteriorly protruding and moderately anterolaterally expanded attachment may be related to a greater contribution of the m. *peroneus longus* in propulsion during plantarflexion. However, the amount of arboreal

locomotion varies among eastern and western gorillas (Remis, 1998). As both species were included and combined in our sample, it is difficult to test this hypothesis properly. More EMG studies are needed to frame better the contribution of peroneal muscles in great ape locomotion behavior.

Distinctive patterns among great apes are also found concerning the m. *soleus* (Figure 2, Figure 6, Figure S5). The 3D-GM analysis shows that all great apes possess a medially expanded insertion of m. *soleus*, which has been linked to their arborealism (Hanna & Schmidt, 2011). African great apes, however, display an inferiorly more protruding m. *soleus* and larger proximal insertion than do orangutans, in agreement with the observed greater mass of this muscle relative to the total m. *triceps surae* mass in gorillas and chimpanzees (Hanna & Schmidt, 2011). This difference may also reflect variation in muscle fiber type distribution (Myatt, Schilling & Thorpe, 2011) and gross muscle anatomy (Marchi *et al.*, 2018; Payne, 2006) among great apes. In particular, it might refer to the need in African great apes for controlled mobility when arboreal and greater speed and power when terrestrial, as compared to the slow movements with a greater joint range of motions that the arboreal behavior of orangutans (Thorpe *et al.*, 1999; Myatt *et al.*, 2011).

A possible limitation of our study is that comparisons among genera may have been affected by differences across species within each genus, which were not addressed in our analyses due to the small sample size. For example, *Pan paniscus* and *Gorilla beringei* are represented by only 1 and 3 specimens, respectively. Indeed, further studies should test for possible differences across species within each genus and possible links to variation in locomotor behavior, as has been shown elsewhere (Doran, 1993; Remis, 1998; Harper, Ruff & Sylvester, 2021).

5. Conclusion

This study expands our understanding of leg structure and posture by providing a detailed description and assessment of proximal fibular shape variation in extant humans and great apes using 3DGM. In addition, our study quantitatively characterized the distinctive features of the human fibular head compared to those of great apes (i.e., oblique articular surface, the presence of the styloid process, and specific morphology of muscle attachment sites),

suggesting a crucial role of the fibula among functional adaptations to human obligate bipedalism.

Great apes also display distinctive features in their proximal fibular morphology, consistent with known differences in locomotor behavior. However, while some features may correlate to the higher degree of arborealism in orangutans (i.e., a laterally expanded m. *peroneus longus* insertion) or contribute to the separation of Asian and African great apes (i.e., the expansion of m. *biceps femoris* and m. *soleus* insertion), others distinct within each genus and might reflect specific hindlimb posture requirements for habitual locomotor behavior (i.e., the orientation of the articular surface). The results of our study provide essential details about the comparative morphology of the proximal fibula in extant hominids that should prove valuable interpretations of fossil hominin locomotor behaviors.

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Data availability statement

Landmarks coordinates and sample lists for geometric morphometric analysis are available in S2 and S3 Appendix. All R scripts utilized for the analyses are available upon request to the corresponding authors.

Conflict of interest

The authors declare no potential conflict of interest.

Author contributions

AP: Conceptualization (equal); formal analysis (lead); investigation (lead); methodology (equal); validation (equal); writing – original draft (lead); writing – review and editing (equal). RS: Formal analysis (supporting); methodology (equal); validation (equal); writing – review and editing (equal). SB: Methodology (equal); resources (equal); supervision (equal); validation (equal); writing – review and editing (equal). MGB: Conceptualization (equal); data curation (equal); funding acquisition (equal); project administration (lead); resources (equal); supervision (equal); validation (equal); writing – review and editing (equal). DM: Conceptualization (equal); data curation (equal); investigation (supporting); resources (equal); supervision (equal); validation (equal); writing – original draft (supporting); writing – review and editing (equal).

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Tables

Taxon	n	Institution	Sex		Side		
			Male	Female	Unknown	Right	Left
<i>Homo sapiens</i>	37	UNIBO	17	20	-	-	37
<i>Pan troglodytes</i>	24	AMNH, NMNH, UZH, SZCM, KUPRI, UNIBO	15	7	2	13	11
<i>Pan paniscus</i>	1	AMNH	-	1	-	-	1
<i>Gorilla gorilla</i>	12	AMNH, UZH, SZCM, KUPRI	8	4	-	7	5
<i>Gorilla beringei</i>	3	AMNH, NMNH	2	1	-	2	1
<i>Pongo pygmaeus</i>	9	AMNH, NMNH, UZH, SZCM,	3	6	-	7	2
<i>Pongo abelii</i>	8	NMNH, UZH, SZCM, KUPRI	7	1	-	5	3

Table 1. Sample composition. Abbreviations: AMNH: American Museum of Natural History (via www.morphosource.org); NMNH: National Museum of Natural History (via www.morphosource.org); UNIBO: Human Identified Skeletal Collection of Bologna; SZCM: State Zoological Collection in Munich; UZH: University of Zurich; KUPRI: Primate Research Institute’s Digital Morphology database

Landmarks	Definition			
L1	Most proximal point of proximal tibiofibular articular facet			
L2	Most proximal point on styloid process of fibular head in medial view			
L3	Most anteroproximal point on anterior border in medial view			
L4	Most posters-proximal point on posteromedial border in medial view			
L5	Most posters-proximal point on posterior border in lateral view (proximal to fibular neck)			
Curves	Definition	N	of	semi-
a	Outline of <i>proximal tibiofibular articular facet</i>	6		landmarks
Surfaces	Definition	N	of	semi-
TFHSur	Proximal <i>tibiofibular articular surface</i>	5		landmarks
Sur	Surface of <i>proximal epiphysis</i>	32		

Table 2. Fibular landmarks and semilandmarks identification, definition, and number.

Features	Genera			
	<i>Gorilla</i>	<i>Pan</i>	<i>Pongo</i>	<i>Homo sapiens</i>
Overall fibular head shape	Squared shape in medial view (anteroposteriorly expanded), with inferiorly projecting, pointed fibular collateral ligament attachment.	Rectangular shape in medial view (anteroposteriorly expanded) with inferiorly projecting, pointed fibular collateral ligament attachments.	Sub-circular shape in medial view (regular proportions anteroposteriorly and mediolaterally) with projecting, pointed collateral ligament attachments.	Squared shape in medial view (regular proportions anteroposteriorly and mediolaterally) with a superiorly raised styloid process (orange arrow) and well-rounded but diminished tibiofibular collateral ligament attachments, located more superiorly.
Anterior border	Well-marked, straight and raised, with an acute border facing anteromedially	Marked and raised with an acute border curving anteriorly at most proximal point	Evident and straight, facing anteromedially, with a more convex border.	Defined and straight, with a convex border facing medially
Medial surface	Moderately concave below the articular border. Some degree of anterior expansion is present, mostly related to the inferior expansion of the m. biceps	Flattened, anteroposteriorly expanded	Marked concavity below the articular border that prolongs to the neck level. The black arrow indicates the anterolateral expansion at the m. peroneus longus	Flattened

	femoris (see below).		attachment area (see below).	
Fibular neck shape	Oval dimensions (anteroposteriorly expanded)	Oval dimensions (anteroposteriorly expanded)	Circular dimensions (regular proportions anteroposteriorly and mediolaterally)	Oval dimensions (anteroposteriorly expanded)
Tibiofibular articular surface	Slightly anteroposteriorly oblique, but approximately horizontal mediolaterally	Horizontal anteroposteriorly and mediolaterally	Slightly anteroposteriorly oblique, but approximately horizontal mediolaterally	Either markedly oblique mediolaterally and slightly anteroposteriorly oblique
Mm. peroneus longus/biceps femoris attachment areas	The attachment area of the biceps femoris tendon is defined and inferiorly expanded (orange arrow), showing a depressed area (imprint, black circle) corresponding to the lateral collateral ligament, facing laterally, while the attachment area of m. peroneus longus, immediately below (black lines) is concave, more	The attachment area of the biceps femoris tendon is defined and inferiorly expanded (orange arrow), showing a slightly depressed area (imprint, black circle) corresponding to the lateral collateral ligament, facing laterally, while the attachment area of m. peroneus longus, immediately below (black lines) is more anterolaterally	The attachment area of the biceps femoris is laterally expanded (orange arrow), showing narrow, deep depression (imprint, black circle), corresponding to the lateral collateral ligament facing laterally. The attachment area of m. peroneus longus, immediately below (black lines) is concave and markedly anterolaterally	The attachment area of the biceps femoris is posteriorly expanded towards the styloid process and rounded anterolaterally (orange arrow). Superiorly, it is present an attenuated depression (imprint, black circle) corresponding to the lateral collateral ligament, facing superiorly. The attachment area of m. peroneus

	anterolaterally facing, expanded laterally in comparison to chimpanzees but not quite as orangutans.	facing but slightly concave.	protruding. This is also evident in medial view (see above), where this area is expanded, quite hollow, and inferiorly elongated	longus (black lines) is narrow and facing anteriorly, with medial expansion and rather flat.
m. soleus attachment area	The area on the insertion is well defined and has a wide horseshoe shape with posteromedial expansion. It is delimited superiorly by a robust posterior projection for the posterior proximal tibiofibular ligament	The area on the insertion is well defined and had has a wide horseshoe shape with posteromedial expansion. It is delimited superiorly by a robust posterior projection for the posterior proximal tibiofibular ligament	The area on the insertion is defined and has a narrow sub-circular shape, medially expanded but less inferiorly elongated. Superiorly, the posterior projection for the posterior proximal tibiofibular ligament is well rounded and not inferiorly elongated.	The area on the insertion has reduced anteroposterior dimensions but is appreciable as a deep horse- shoe groove, facing posteriorly. It is elongated proximo-distally. It is delimited superiorly by a robust posterior projection for the posterior proximal tibiofibular ligament that culminates superiorly into the styloid process

Table 3. Summary of the relevant morphological features observed in the four genera analysed (*Homo*, *Pan*, *Gorilla* and *Pongo*).

PC2	PC1			
	Pan	Gorilla	Pongo	H. sapiens
Pan	-	0.060	0.000	0.000
Gorilla	0.000	-	0.140	0.000
Pongo	0.000	0.434	-	0.000
H. sapiens	0.001	0.000	0.000	-

Table 4. Pairwise *t*-test on principal components (PC) 1 and PC2 scores *Pan*, *Gorilla*, *Pongo* and *Homo sapiens*. Results in bold are statistically significant. Results above the diagonal represent group comparisons along PC1, while results below the diagonal represent group comparisons along PC2.

	Gorilla	Pongo	H. sapiens
Pan	0.011	0.001	0.000
Gorilla	-	0.136	0.000
Pongo		-	0.000

Table 5. Post-hoc pairwise comparisons among *Pongo*, *Gorilla*, *Pan* and *Homo sapiens* after Procrustes ANOVA. Results in bold are statistically significant.

PC2	PC1		
	Pan	Gorilla	Pongo
Pan	-	0.000	0.000
Gorilla	0.238	-	0.015
Pongo	0.343	0.023	-

Table 6. Pairwise *t*-test on principal components (PC) 1 and PC2 scores of *Pan*, *Gorilla* and *Pongo*. Results in bold are statistically significant. Results above the diagonal represent comparisons along PC1, while result below the diagonal represent comparisons along PC2.

	<i>Pan</i>	<i>Gorilla</i>	<i>Pongo</i>
<i>Pan</i>	-	0.001	0.001
<i>Gorilla</i>		-	0.032
<i>Pongo</i>			-

Table 7. *Post-hoc* pairwise comparisons among great apes after Procrustes ANOVA. Results in bold are statistically significant.

Figures

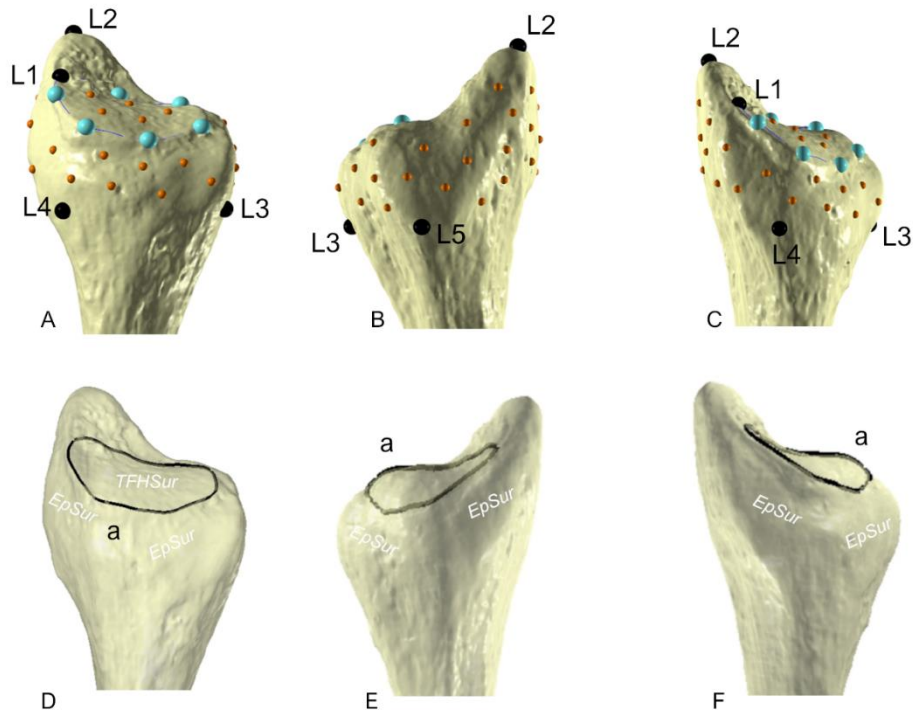


Figure 1. Landmark configuration on a human left proximal fibula. (A), proximal view, (B) anterolateral view and right, (C) posteromedial view. Black dots are fixed landmarks, light blue dots are curve semilandmarks and orange dots are surface semilandmarks. (D) curve "a" definition on a human left proximal fibula, proximal view. See Table 2 for definition of numbered landmarks and curve. EpSur= epiphyseal surface; TFHSur= tibiofibular articular surface.

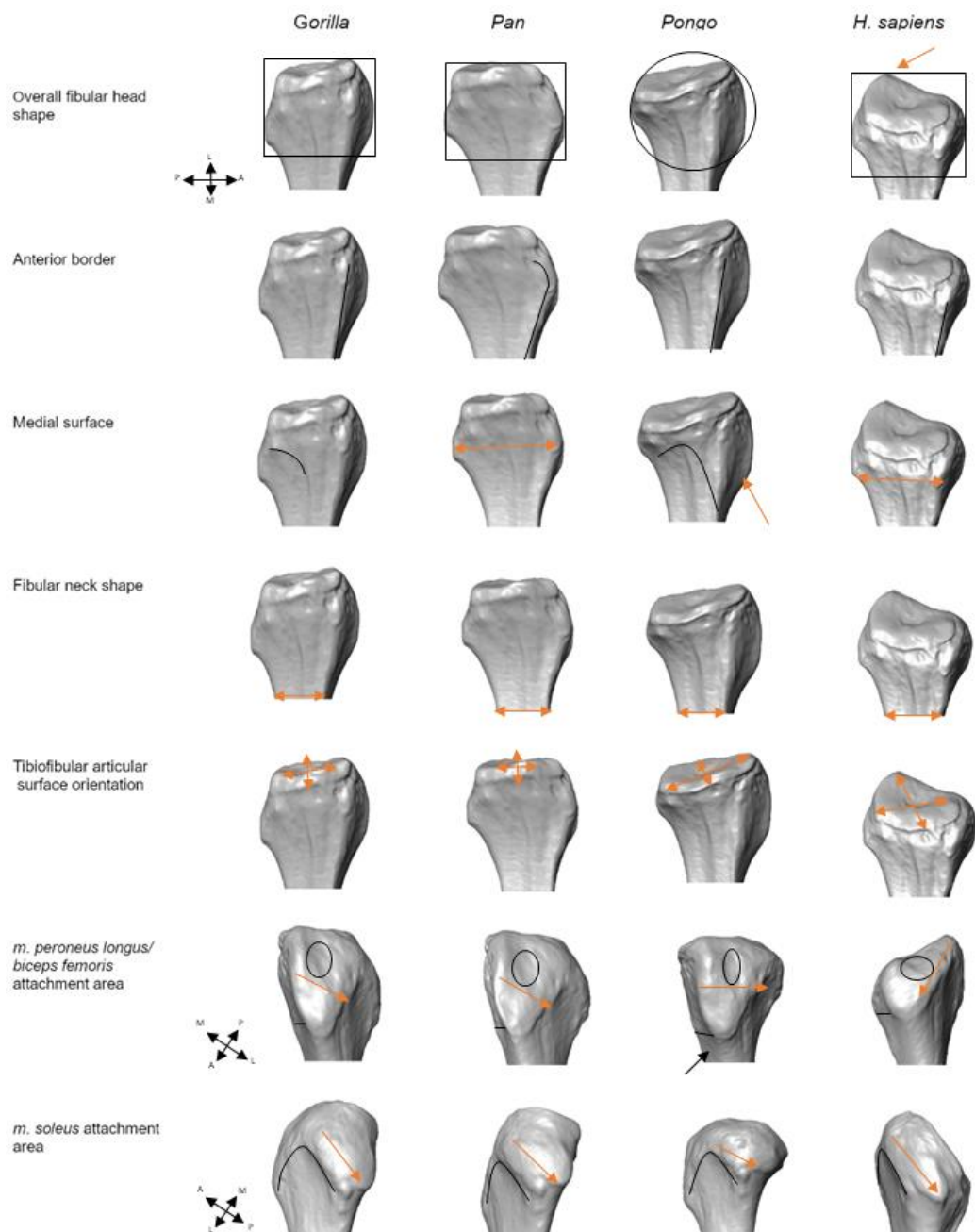


Figure 2. Schematic representation of major morphological differences observed at proximal left fibula in *Gorilla*, *Pan*, *Pongo* and *Homo sapiens*, with site specific detailing. Fibular morphings refer to extreme morphings configurations obtained for each genus along the first two principal components. The first five rows show fibulae in medial view, the last two show fibulae in antero-lateral and postero-lateral view, respectively. At the top row, shapes indicate the overall morphology of fibular head. At the second row, curved lines indicate the outline of the anterior border. The third row, arrows and curved lines represent respectively the concave or flat medial surface. Arrow in the fourth row show the thickness of fibular neck. At fifth row, orange arrows show the orientation of PTFJ. At sixth row, black empty ellipses indicate the FCL insertion, orange arrows show the projection of the PL/BT area, black lines show the antero-lateral expansion of the PL, and black arrows show the lateral expansion of the PL. At bottom row, black lines show the outline of m. soleus insertion, orange arrows indicate the inferior projection of the area of insertion of PPTL. PTFJ: proximal tibio-fibular joint; PPTL: anterior proximal tibiofibular ligaments; FCL: fibular collateral ligament; BT: m. biceps femoris tendon attachment; PL= m. peroneus longus origin.

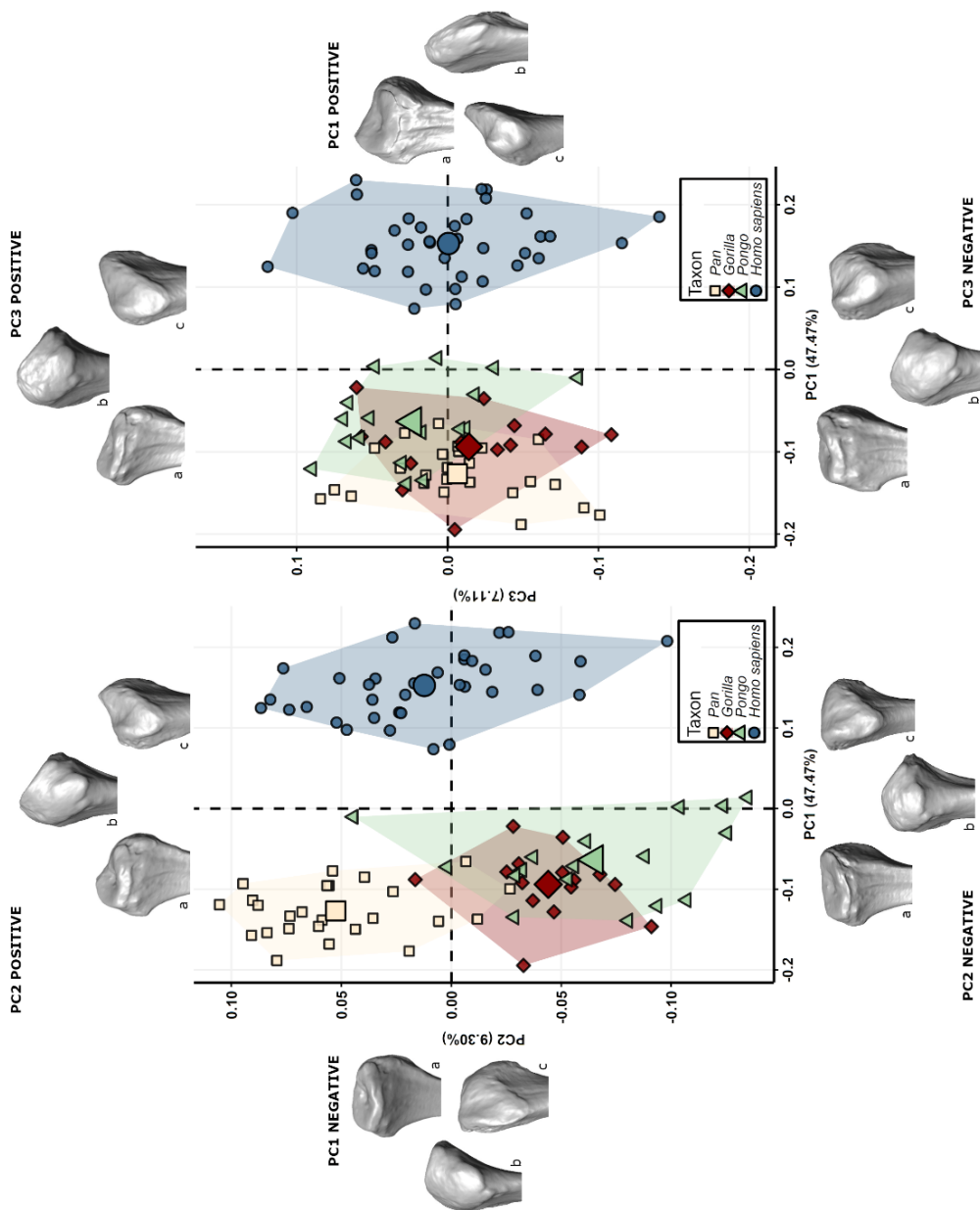


Figure 3. Shape space scatterplots of second versus first component (PC2 vs. PC1, on the left) and third versus first principal component (PC3 vs. PC1, on the right, B) of Pan (yellow squares), Gorilla (red rhombuses), Pongo (green triangles) and Homo sapiens (blue circles) and relative means, with shape space extreme morphings in medial (a), posterior (b) and antero-lateral (c) views of a left fibula. PC1 extreme positive and negative morphings are displayed on the right and the left, respectively. PC2 extreme positive and negative morphings are displayed at left top and left bottom, respectively, while PC3 extreme positive and negative morphings are displayed at right top and right bottom.

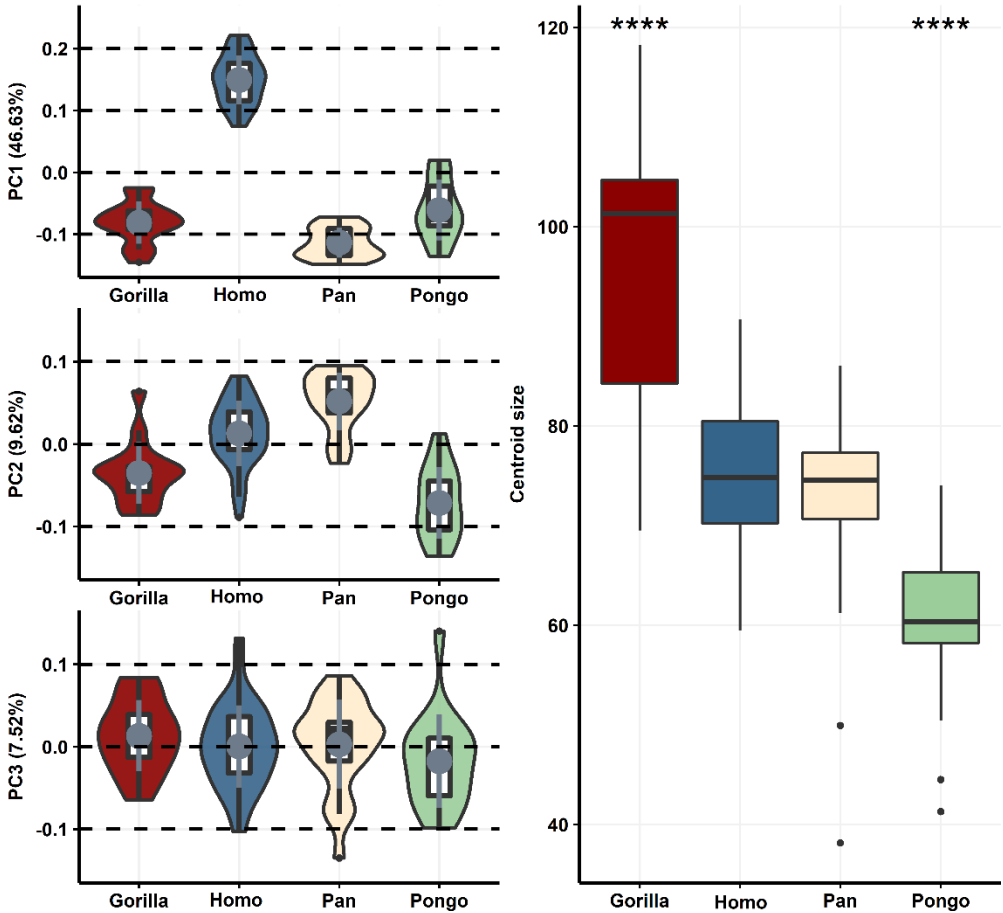


Figure 4. On the left, violin plots representing the distribution of principal component (PC) scores along the first three PC of *Pongo*, *Gorilla*, *Pan* and *Homo sapiens*. Gray circles are the medians, black boxes represent the interquartile ranges, whiskers the non-outliers range. On the right, box plots representing centroid size variations of *Pongo*, *Gorilla*, *Pan* and *H. sapiens*. Black lines are the medians, black boxes represent the interquartile ranges, whiskers the non-outliers range and black circles the outliers. Asterisks show ANOVA post-hoc significant comparisons of centroid size among groups.

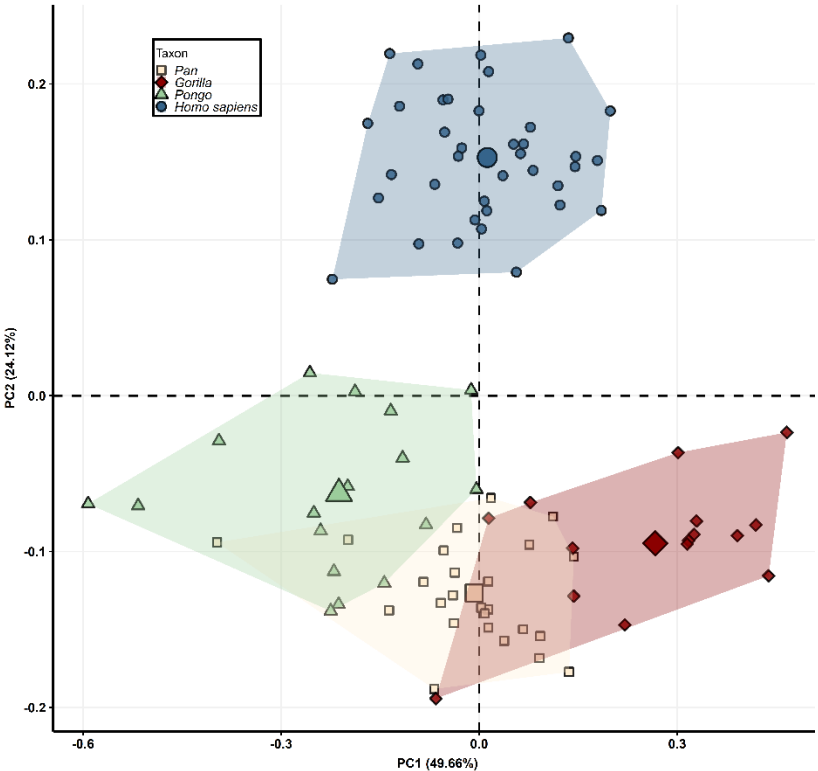


Figure 5. Form space principal component analysis scatterplots considering principal component (PC) scores of *Pan* (yellow squares), *Gorilla* (red diamonds), *Pongo* (green triangles) and *Homo sapiens* (blue circles) and relative group means along the first two PCs.

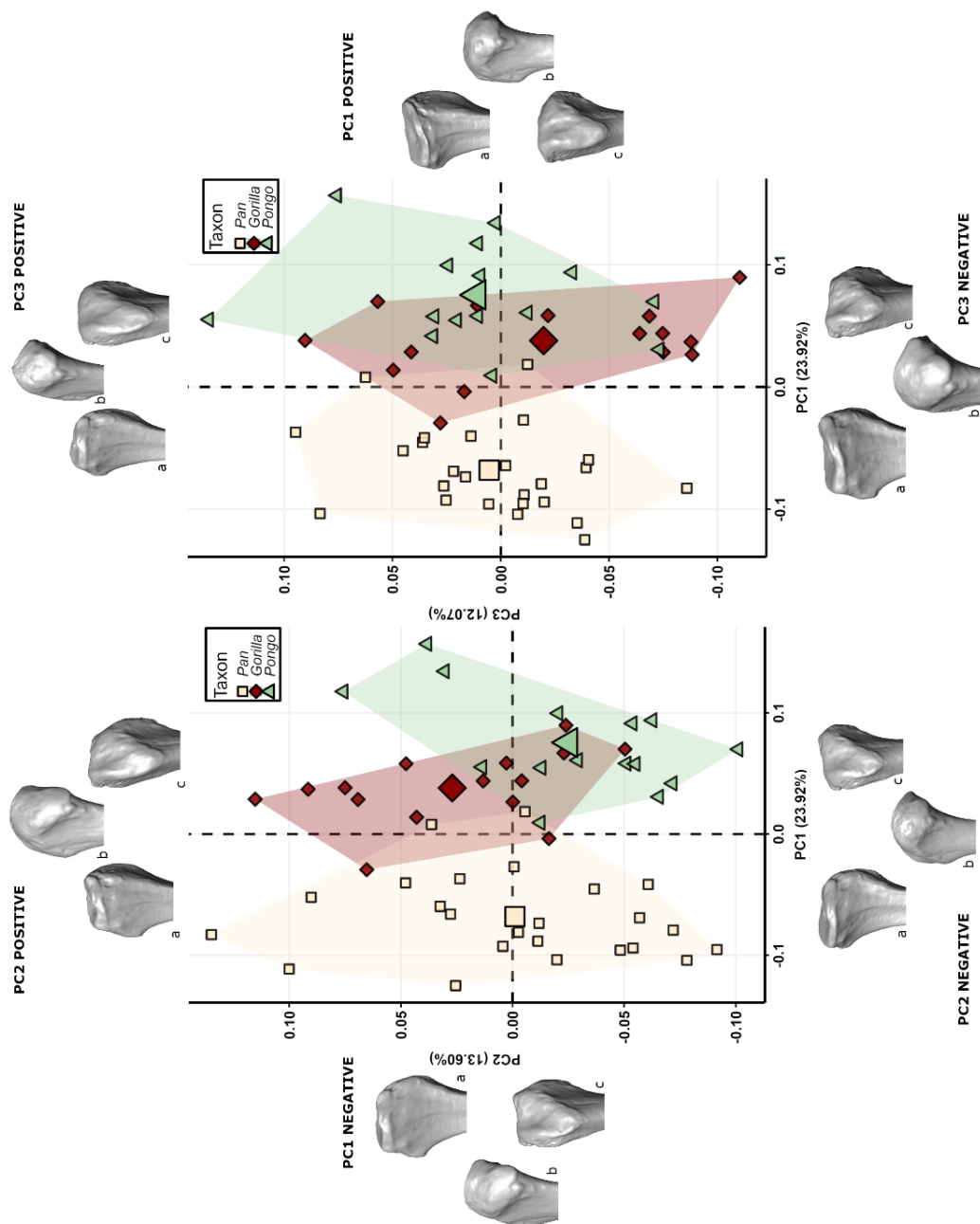


Figure 6. Shape space scatterplots of second versus first component (PC2 vs. PC1, on the left) and third versus first principal component (PC3 vs. PC1, on the right) of Pan (yellow squares), Gorilla (red rhombuses) and Pongo (green triangles) and relative means, with shape space extreme morphings in medial (a), posterior (b) and antero-lateral (c) views of a left fibula. PC1 extreme positive and negative morphings are displayed on the right and the left, respectively. PC2 extreme positive and negative morphings are displayed at left top and left bottom, respectively, while PC3 extreme positive and negative morphings are displayed at right top and right bottom.

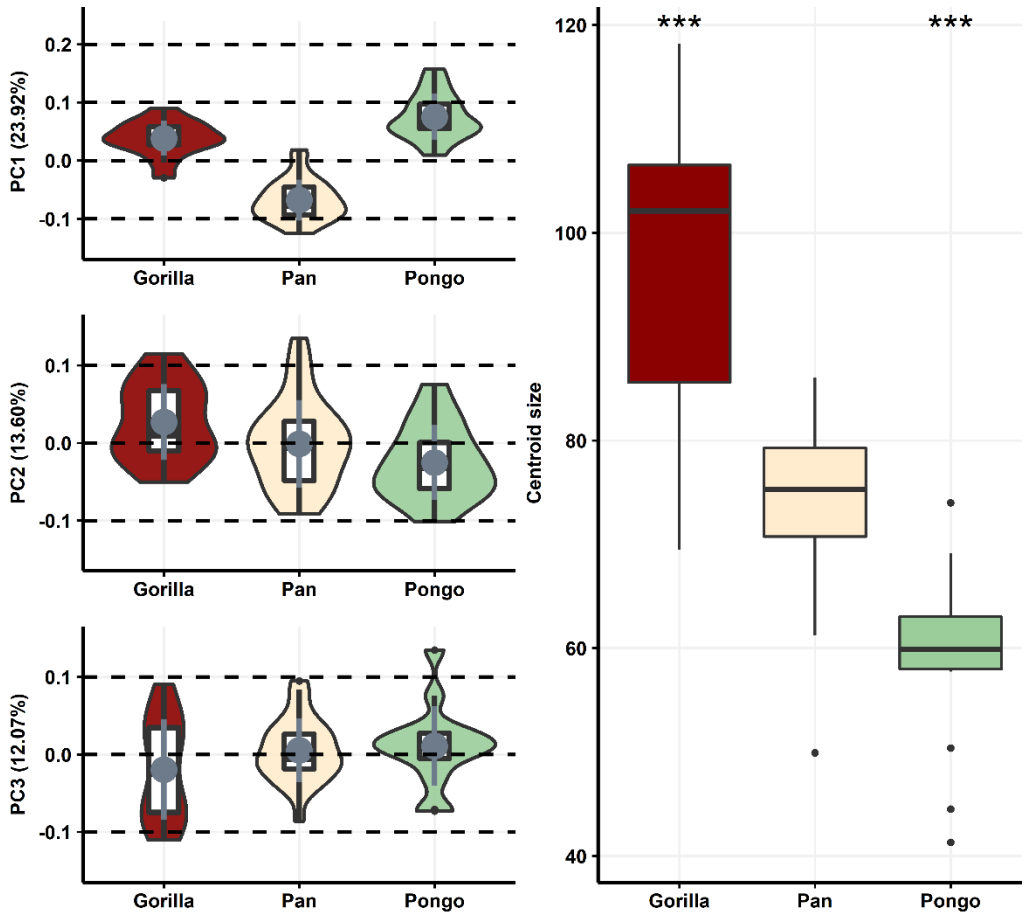


Figure 7. On the left, violin plots representing the distribution of principal component (PC) scores along the first three PCs of *Pongo*, *Gorilla* and *Pan*. Gray circles are the medians, black boxes represent the interquartile ranges, whiskers the non-outliers range. On the right, box plots representing centroid size variations of *Pongo*, *Gorilla* and *Pan*. Black lines are the medians, black boxes represent the interquartile ranges, whiskers the non-outliers range and black circles the outliers. Asterisks show ANOVA post-hoc significant comparisons of centroid size according to groups.

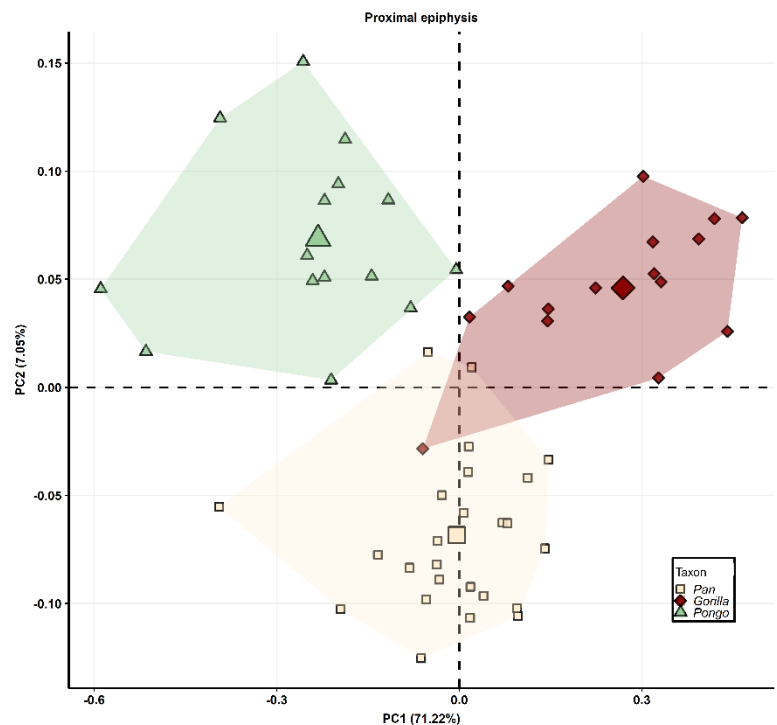


Figure 8. Form space PCA scatterplots considering PC scores of PC scores of *Pan* (yellow squares), *Gorilla* (red rhombuses) and *Pongo* (green triangles) groups and relative groups' means along the first two PCs.

Supplementary materials are available at:
<https://onlinelibrary.wiley.com/doi/10.1002/ajpa.24696>



REPORT

Distal fibular epiphysis in living hominids

CONTENTS

- REPORT ON
RESULTS FOR
DISTAL EPIPHYSIS
IN HOMINIDS

Result report on distal epiphysis in great apes

This section presents the preliminary results on distal fibular shape in living hominids, that are currently under investigations.

The shape PCA plot (PC1= 58.28%; PC2 = 8.94 %; PC3 = 8.38%) (Figure 1) clearly shows the separation along PC1 between *H. sapiens*, plotting towards PC1 positive scores, and great apes, plotting towards PC1 negative scores. Figure 2 shows the shape morphings obtained. PC1 positive extreme shape, characterizing *H. sapiens*, is mostly driven by vertically oriented, medially facing proximal and distal talofibular articular surfaces (PAF and DAF), an elongated and smooth interosseous ligament attachment (ILA), a downward facing, pointed malleolus, a laterally facing subcutaneous triangular surface (STS), and narrow, less concave peroneal grooves and malleolar fossae. PC1 negative extreme shape, characterizing great apes, is mostly driven by tilted downward facing DAF with a medially facing PAF, a shortened and rough interosseous ligament attachment (ILA), a laterally facing, rounded malleolus, a anteriorly facing STS, and wide, concave peroneal grooves and malleolar fossae. All groups diverge from one another in terms of Procrustes distances (p -value < 0.01) (Table 1), except for gorillas and chimpanzees. Significant size different emerged among groups, with orangutans and humans significantly differing from the other groups, being the smallest and largest groups respectively.

Great apes show distinct features, such as bulkier insertions and more mobile articular surfaces than humans: a more robust ILA, seen in highly nonhuman hominids, may facilitate the increase of load-sharing by the fibula, likely occurring in vertical climbing and clambering, or traversing uneven terrain (Rantalainen *et al.*, 2010; Sparacello, Marchi & Shaw, 2014), while also ensuring fibular stabilization (Minns and Hunter, 1976; Skraba and Greenwald, 1984; Thomas, *et al.*, 1995). Moreover, the distal talofibular articular surfaces orientation may reflect a more everted foot, with a medio-laterally wider motion range (Barnett & Napier, 1952).

Our study quantitatively characterized the distinctive features of the human fibular ends in comparison to those of great apes (i.e., vertical and medially facing talofibular articulations, a lateral STS with a pointed and downward

facing malleolus, specific morphology of muscle attachment sites) suggesting a crucial role of the fibula among functional adaptations to human obligate bipedalism.

Tables

	<i>Gorilla</i>	<i>Pongo</i>	<i>H. sapiens</i>
<i>Pan</i>	0.0658	0.0030	0.0001
<i>Gorilla</i>	-	0.0323	0.0001
<i>Pongo</i>		-	0.0001

Table 1. *Post-hoc* pairwise comparisons among *Pongo*, *Gorilla*, *Pan* and *Homo sapiens* after Procrustes ANOVA. Results in bold are statistically significant.

Figures

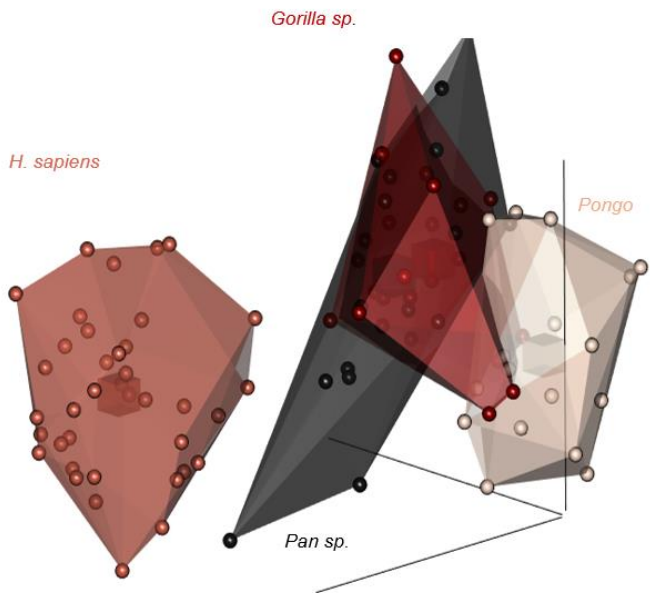


Figure 1. 3D shape space PCA plot displaying distribution of individuals according to distal fibular shape variation among genera. PC1= 58.28%; PC2 = 8.94 %; PC3 = 8.38%.

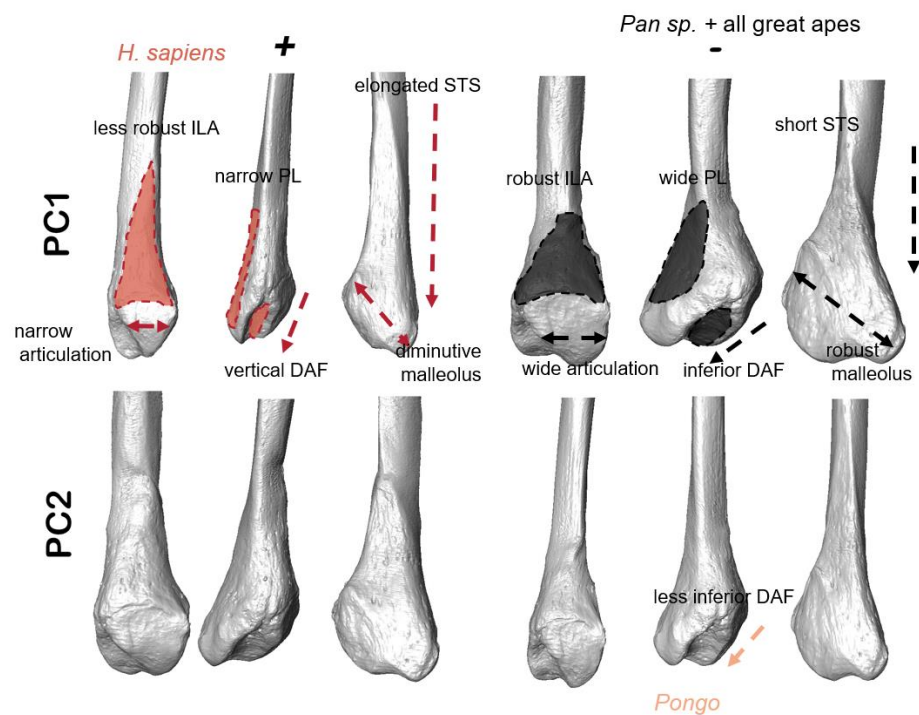


Figure 2. Distal fibular shape extreme shapes reconstruction among PC1 and PC2 according to genera with a summary description of morphological variations.



CHAPTER 4

Final remarks

CONTENTS

- CONCLUSIONS
 - PERSPECTIVES
-

4. Final remarks

This thesis explored fibular shape and form epiphyseal variation in extant hominids, documenting significant functional aspects that linked fibular morphology to relevant postural behavior, further supporting the form-function relationship that is assumed by the model known as “bone functional adaptation”.

This work detailed patterns of fibular morphological changes in humans in relation to intraspecific variations linked to sexual dimorphism, to interpopulation variation linked to subsistence and mobility and in hominid taxa in relation to interspecific variations linked to phylogeny and locomotor behavior.

Regarding fibular shape in humans in relation to **intraspecific variations linked to sexual dimorphism**, our study was first to address fibular epiphyseal shape, form, and size in relation to sexual dimorphism adopting a three dimensional geometric morphometrics (3DGM) method that includes both fixed landmarks and sliding (semi)landmarks, that together account for detailed morphological variations of fibular extremities. We highlighted sex-related differences in fibular form that mainly consist in larger and more robust epiphyses in males than in females, with females showing narrower articular surfaces, shorter and narrower malleoli, shallower malleolar fossa and peroneal groove, and less protruding muscle insertions for the m. *fibularis longus*, m. *biceps femoris* and interosseous ligament distal attachment. This form differentiation among males and females may reflect a sex-specific pattern of knee and ankle/foot posture, with distinctive lower limb morpho- functional aspects in relation to sex. Additionally, we provided a novel methodology that can be used to determine sex in both bioarchaeological and forensic investigations using the proximal and distal extremities of the human fibula. A different degree of sexual dimorphism in populations with different ancestries, that is not strictly related to size variations, was additionally highlighted.

Regarding fibular shape in humans in relation to **interpopulation variations linked to changes in subsistence strategy and mobility**, our results reveal the potential of fibular epiphyseal morphology as indicator of mobility patterns. We highlighted shape changes of fibular proximal and distal ends among Italian

populations dated from the Mid-Late Upper Paleolithic to the 20th century through a 3DGM approach. We found a pattern consistent with a progressive decline of activity level associated to the type of terrain and shoe use, especially evident when comparing hunter-gatherers to sedentary pre- and post-industrialists. Some fibular traits (e.g., horizontal proximal tibiofibular and tilted distal talofibular articular surfaces, robust interosseous membrane attachment, broad malleolar fossa, projecting m. *biceps femoris*) indicate greater mobility of fibular joints and degree of load sharing by the fibula during an anteroposterior excursion of the knee and ankle in highly active populations, compared to more rigid fibular joints in sedentary populations. These morphologies may be related to habitual and frequent ankle and knee flexion that is consistent with different foraging subsistence strategies, such as a combination of frequent traversing of uneven terrain with no foot coverings in highly active populations, and restricted ankle excursion by limited lower limb loading and use in plain urban settlements with hard-shoe coverings in sedentary populations. Other fibular traits (e.g., shorter STS, projecting malleolus, concave peroneal groove, and proximal insertion) might indicate for mobile groups a more everted foot posture with increased moment arm in eversion, more efficient for barefoot running in forefoot-strike gait and concurring in the stabilization of the first metatarsophalangeal joint.

Regarding fibular shape in relation to **interspecific variation among living hominids linked to locomotor behavior**, we documented fibular morphological variation with functional significance among hominid taxa, possibly in relation to both phylogeny and specific loading patterns on the lower limb. Our study quantitatively characterized the distinctive features of the human fibular ends in comparison to those of great apes (i.e., oblique proximal articular surface, vertical and medially facing talofibular articulations, the presence of the styloid process and a lateral STS with a pointed and downward facing malleolus, specific morphology of muscle attachment sites) suggesting a crucial role of the fibula among functional adaptations to human obligate bipedalism. Great apes also display distinctive features in their fibular morphology, consistent with known differences in locomotor behavior compared to humans (i.e., mobile horizontal proximal articular surface and

inferiorly facing talofibular articulations, anteriorly oriented STS with a robust malleolus, robust muscle attachment sites). Some of the observed features correlate to the higher degree of arborealism in orangutans (i.e., a laterally expanded m. *peroneus longus* proximal and distal insertion; wide ankle collateral ligaments, distal articular surface) or contribute to the separation of Asian and African great apes (i.e., the expansion of m. *biceps femoris* and m. *soleus* insertion). Other traits might reflect specific hindlimb posture requirements for habitual locomotor behavior (i.e., the orientation of the proximal articular surface in gorillas and orangutans).

Our study documents the importance of considering fibular epiphyseal morphology in relation to loading patterns. More kinematic data obtained in clinical/experimental setups for both humans and great apes are needed to further test the form-function relationship between specific fibular morphologies as specific motions and mechanisms.

The results of our study provide important details about the comparative morphology of the proximal and distal fibula in extant hominids that will prove useful for the interpretation of fossil hominins locomotion behavior in an evolutionary perspective.

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