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Posted Date: 21 November 2025

doi: 10.20944/preprints202511.1652.v1

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Article

Stepping Up: Accessory Bones of the Foot in the 21st Century Identified Skeletal Collection (Portugal)

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Abstract

The anatomical variability of the human foot represents a subject of substantial interest, offering valuable insights in anthropological research as well as in clinical practice. The aim of this study is to document anatomical variants of the tarsal bones in the CISC//XXI skeletal sample (21st Century Identified Skeletons Collection), with a particular focus on the prevalence of accessory ossicles. The studied sample consisted of 163 individuals (83 female and 80 males). The prevalence of six accessory tarsal bones was registered (*os trigonum*, *calcaneum secundarium*, the accessory navicular bone, *os sustentaculum*, *os vesalianum* and *os intermetatarseum*). A total of 35 individuals (21.4%; 35/163) exhibited at least one accessory ossicle, 13 females (15.7%; 13/83) and 22 males (27.5%; 22/80). The *os trigonum* and *calcaneum secundarium* were the most frequently observed accessory bones, respectively in 9.3% (15/162) and 6.9% (11/159) of the individuals. No sex differences were observed. All the accessory bones occurred more frequently unilaterally, and no co-occurrences of accessory bones were observed. These findings are crucial in both the biomedical and anthropological fields, where a detailed knowledge of foot anatomy and its variations is relevant.

Keywords: foot anatomical variants; tarsal bones; accessory ossicles; identified skeletal collection

1. Introduction

Morphological variations in the human skeleton, particularly those observed in accessory bones (secondary ossicles), fusion defects, and other rare anatomical anomalies, are of notable interest in anthropological and clinical contexts [1–3]. The study of foot bones enables anthropologists to identify unique skeletal characteristics, such as anatomical variants and fusion patterns. When these characteristics are correlated with clinical evidence, they can be used to ascertain individual identity [4]. In clinical terms, such analyses can also provide insights into locomotor function, congenital or developmental anomalies, degenerative changes, and trauma patterns. Variations in foot morphology include bipartitions, bony fusions, sesamoid bones, and accessory ossicles [2,5,6].

A comprehensive understanding of tarsal anomalies is of paramount importance for both clinicians and orthopedical researchers, as it offers invaluable insights into a multitude of aspects, including clinical assessments, diagnostic processes, and treatment planning [1,2,7–9]. Anatomical variants in the tarsal region frequently manifest as deviations in the number, shape, size, or position of the bones [10], being often identified fortuitously, which complicates the process of determining their etiology and morphological significance.

Accessory ossicles, also known as accessory or supernumerary bones, represent skeletal variations that are typically small in size. While often asymptomatic, they can occasionally be associated with pain or functional impairment [2,11]. These ossicles can occur as additional independent elements or as abnormal subdivisions of existing bones [12,13]. These subdivisions have their origin in either the incomplete fusion of the secondary ossification centers or the atypical

division of the primary ossification centers [1]. The presentation of accessory ossicles is heteroclit, manifesting as unilateral or bilateral formations. In certain cases, the ossicles can fuse with adjacent bones [14].

Early research focused on foot anatomical variants relied heavily on cadaveric dissections, which provided critical insights into their anatomy and prevalence [10,15–19]. Although symptomatic ossicles are considered uncommon, recent literature indicates an increasing number of cases describing associated pain syndromes and degenerative changes [2,9,11,12,14,20–23].

The reported prevalence of accessory ossicles of the foot varies considerably across studies, that likely stem from multiple biases, as highlighted by Silva and Curate [3], and include differences in the type of sample or imaging technique, the non-detection of asymptomatic cases in clinical settings, and inconsistencies in prevalence estimation (per foot or per individual).

The aim of the present study is to record the frequency of six accessory foot ossicles (*os trigonum*, *os calcaneum secundarium*, navicular accessory bone, *os sustentaculum*, *os vesalianum*, and *os intermetatarsale*) in a Portuguese sample from the 21st Century Identified Skeletal Collection (CISC/XXI). In addition to frequency, patterns of co-occurrence, laterality, and potential differences by biological sex and age were also assessed.

2. Materials and Methods

Study Sample

The analysis was conducted on the 21st Century Identified Skeleton Collection (CISC/XXI), housed at the University of Coimbra (Forensic Anthropology Laboratory, Department of Life Sciences, Coimbra, Portugal). This collection consists of 302 adult skeletons (162 females; 140 males) from the *Cemitério dos Capuchos* (Santarém), belonging to individuals exhumed between 1999 and 2016. All individuals died between 1982 and 2012 [24,25].

The study sample consisted of 162 adult individuals (82 females; 80 males), with ages at death ranging from 25 to 101 years old (average age at death = 75.0 years; SD=15.8).

The Accessory Ossicles of the Foot

The nature of the sample, i.e., dry bone, constrained the selection of the accessory bones, since it is impossible to fathom if every small accessory and sesamoid bones were collected during excavation. Therefore, six accessory foot bones were included: *os trigonum*, *os calcaneum secundarium*, navicular accessory bone, *os sustentaculum*, *os vesalianum*, and *os intermetatarsale*. Operational definitions for these ossicles follow Silva and Curate [3].

Statistical Analysis

The frequencies of each accessory ossicle were calculated, and reported as prevalences in percentage (per individual and per foot). A chi-square test of independence (X^2) was conducted under the null hypothesis that the proportion of individuals with an accessory ossicle is the same in both sexes. Laterality was also assessed with a chi-square test. A student's t-test (independent samples) was employed to assess the null hypothesis that the average age at death is identical in individuals with and without accessory foot ossicles. Normality of the sample was evaluated with a Q-Q plot, and homoscedasticity with a Levene's test. The critical p-value for rejecting the null hypothesis was set at $p < 0.05$. All statistical analyses were performed using Jamovi (v. 2.3.28).

3. Results

Of the 162 skeletons analyzed, 35 individuals (21.6%; 35/162), 13 females (15.9%; 13/82) and 22 males (27.5%; 22/80) exhibit at least one accessory ossicle. There is no statistically significant association between sex and the presence of accessory foot bones (X^2 : 2.082, $df=1$, $p=0.149$). A small difference was recorded in the mean ages at death of individuals with (mean = 71.5 years old; SD =

16.9) and without (mean = 76.1 years old; SD = 15.4) accessory ossicles, but the difference is not statistically significant (t-test: 1.55, df = 161, p = 0.123).

No co-occurrence of accessory foot bones was recorded. The prevalence of the observed ossicles of the foot per individual is summarized in Table 1. Similarly, the prevalence of ossicles in each foot is shown in Tables 2 and 3.

Table 1. Prevalence of foot ossicles per individual in the CISC/XXI sample (n: individuals with foot ossicles, N: sample size).

	Females		Males		Total	
	Prevalence	n/N	Prevalence	n/N	Prevalence	n/N
<i>Os trigonum</i>	8.5	7/82	10.0	8/80	9.3	15/162
<i>Calcaneum secundarium</i>	3.8	3/80	10.1	8/79	6.9	11/159
Accessory navicular bone	0.0	0/80	1.3	1/80	0.6	1/160
<i>Os sustentaculum</i>	0.0	0/80	0.0	0/80	0.0	0/160
<i>Os vesalianum</i>	1.3	1/80	0.0	0/79	0.6	1/159
<i>Os intermetatarsium</i>	2.5	2/79	6.6	5/76	4.5	7/155

Table 2. Prevalence of foot ossicles in the CISC/XXI sample (left foot; n: individuals with foot ossicles, N: sample size).

	Females		Males		Total	
	Prevalence	n/N	Prevalence	n/N	Prevalence	n/N
<i>Os trigonum</i>	6.3	5/79	8.0	6/75	7.1	11/154
<i>Calcaneum secundarium</i>	1.3	1/77	5.2	4/77	3.2	5/154
Accessory navicular bone	0.0	0/74	0.0	0/69	0.0	0/143
<i>Os sustentaculum</i>	0.0	0/76	0.0	0/77	0.0	0/153
<i>Os vesalianum</i>	0.0	0/73	0.0	0/70	0.0	0/143
<i>Os intermetatarsium</i>	2.7	2/73	2.9	2/70	2.8	4/143

Table 3. Prevalence of foot in the CISC/XXI sample (right foot; n: individuals with foot ossicles, N: sample size).

	Females		Males		Total	
	Prevalence	n/N	Prevalence	n/N	Prevalence	n/N
<i>Os trigonum</i>	6.4	5/78	5.2	5/77	6.5	10/155
<i>Calcaneum secundarium</i>	3.8	3/78	7.8	6/77	5.8	9/155
Accessory navicular bone	0.0	0/74	1.3	1/76	0.7	1/150

<i>Os sustentaculum</i>	0.0	0/80	0.0	0/80	0.0	0/160
<i>Os vesalianum</i>	1.3	1/80	0.0	0/79	0.6	1/159
<i>Os intermetatarsium</i>	2.5	2/79	6.6	5/76	4.5	7/155

Os Trigonum

The most frequently observed accessory bone was *os trigonum*, present in 15 individuals (9.3%, 15/162; Table 2). The prevalence is 8.5% in females (7/82) and 10.0% in males (8/80), with no significant differences by sex (X^2 : 0.745, $df=1$, $p=0.388$). Six individuals (40%, 6/15), three females and three males, present with the ossicle bilaterally (Figure 1).



Figure 1. Inferior view of the left and right talus, respectively, of a female individual (CISC/XXI29) with *os trigonum*. The left bone exhibits a fused ossicle, while the right bone presents a free ossicle.

Of the 15 individuals presenting with this ossicle, 14 (93.3%; 14/15) exhibit a fused *os trigonum*. Two individuals (a male and a female) show bilateral fusion of this ossicle (Figure 2). A unilateral fusion of this accessory bone was recorded in six males (three on the left foot and three on the right foot) and six females (four on the left foot and two on the right foot) (Figure 3).

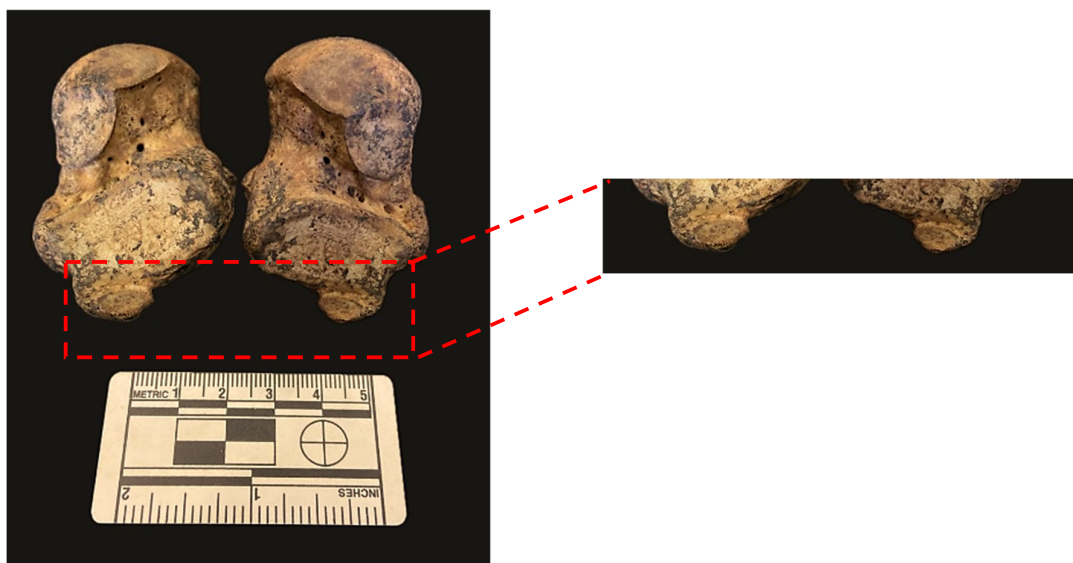


Figure 2. Inferior view of the left and right talus of a female individual (CISC/XXI/299) illustrating bilateral, fused, *os trigonum*.

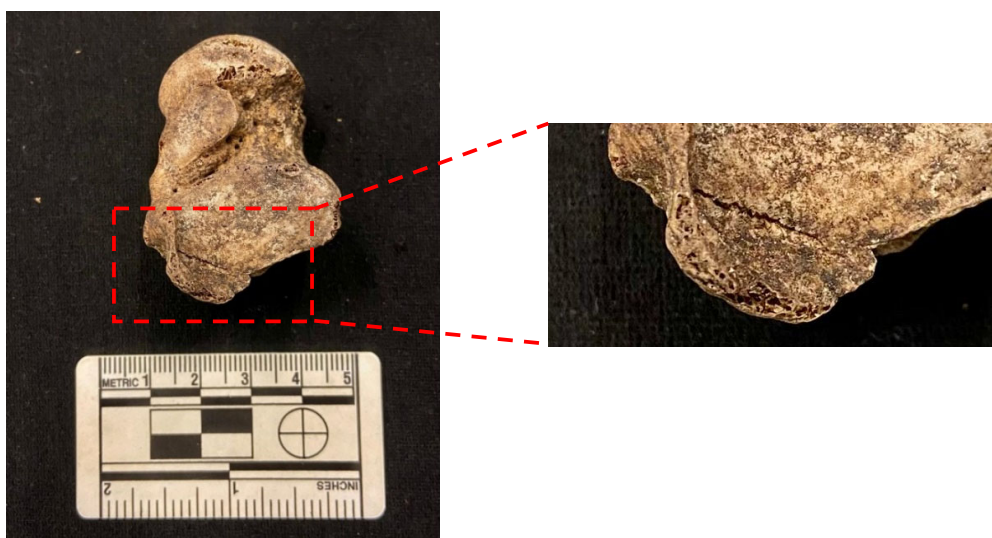


Figure 3. Inferior view of the left talus of a female individual (CISC/XXI/59) with unilateral fusion of *os trigonum*. Note the fusion line in the close up.

The prevalence of this ossicle is 7.1% (11/154) for the left foot (Table 2), 6.3% in females (5/79) and 8.0% in males (6/75). Five of the affected women (100%, 5/5) and four men (66.7%, 4/6) present with a fused *os trigonum* on the left foot. In the right foot, the prevalence is 6.5% (10/155), five females (6.4%, 5/78) and five males (6.5%, 5/77, Table 3). Three females (60.0%; 3/5) and four males (80.0%; 4/5) show a fused *os trigonum* in the right foot. This ossicle was observed bilaterally in 40% (6/15) of the individuals. Five (55.6%; 5/9) unilateral cases were found in the left foot and four (44.4%; 4/9) in the right foot. Only one free ossicle was detected, specifically in the right foot of a male subject (Figure 4).

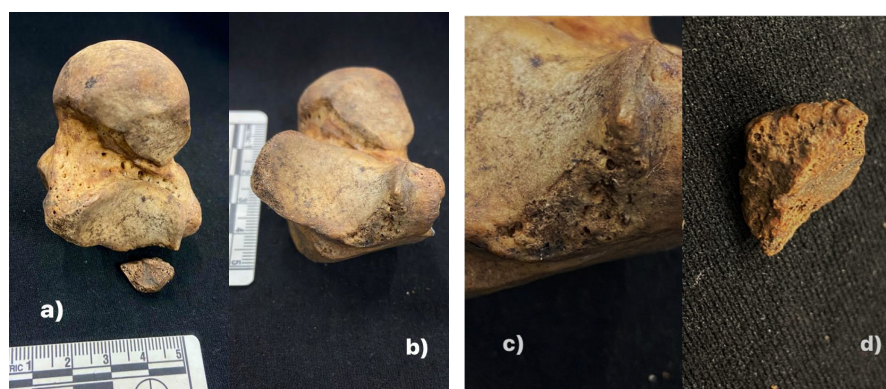


Figure 4. a) and b) Inferior view of the right talus of a male individual (CISC/XXI/114) with the ossicle free; c) Infero-medial view. Note the triangular shape and d) The free *os trigonum*.

Calcaneum Secundarium

Calcaneum secundarium (CS) is the second most prevalent ossicle observed in this study, with a total prevalence per individual of 6.9% (11/159) (Table 1). The ossicle is present in three females (3.8%; 3/80) and eight males (10.1%; 8/80), but the difference between sexes is not statistically significant (X^2 : 2.51; $df=1$; $p=0.113$). Three individuals, one female and two males, exhibit the ossicle bilaterally (27.3%; 3/11; Figure 5).

On the left foot, the prevalence is 3.2% (5/154, Table 3), 1.3% in females (1/77) and 5.2% in males (4/77). The prevalence in the right foot is 5.8% (9/155, Table 4), 3.8% in females (3/78) and 7.8% in males (6/77). This accessory bone is expressed bilaterally in 27.3% of individuals. The majority of

unilateral cases (75%; 6/8) were observed in the right foot, while only two left unilateral cases were recorded (25%; 2/8) (Figure 6).

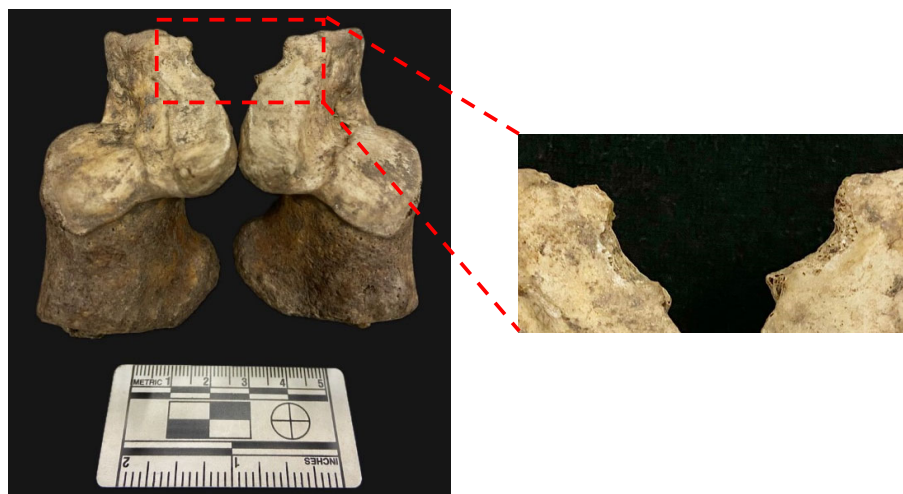


Figure 5. Bilateral case of *calcaneum secundarium* in a male individual (CISC/XXI/109). Detailed view of the anteromedial aspect of the calcaneus. Note that the anterior and medial facets are continuous.



Figure 6. Superior view of the right calcaneus of a male individual (CISC/XXI/252). The anterior and medial facets are separated.

A statistically significant association was observed between laterality and the presence of *calcaneum secundarium* ($X^2: 39.2, df=1, p < 0.001$), with a higher prevalence in the right foot. The most common location of this ossicle is on the anterior facet of the calcaneus, specifically on the anteromedial aspect. Of the nine calcanei with CS, five exhibit separate facets, while the remaining four show a continuous facet.

Os sustentaculum

This ossicle was not identified in any of the 163 individuals under study.

Os Vesalianum

This accessory bone was observed only in the right foot of one female (CISC/XXI/25), (0.6%, 1/159, Tables 1 and 3).

Accessory navicular bone

A Type II accessory navicular was identified in the right foot of one male (CISC/XXI/72) (0.6%, 1/160; Tables 1 and 3; Figure 7).

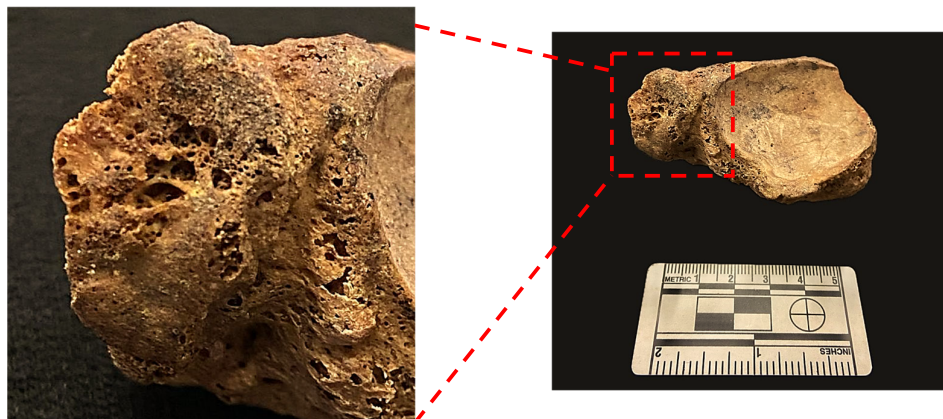


Figure 7. Right navicular bone of a male individual (CISC/XXI/72), showing a unilateral case of Type II accessory navicular bone. On the close-up (left), note the flattened and porous surfaces of the bone.

Os intermetatarsium

Only fused cases of this small ossicle were detected. This ossicle was identified in seven individuals (4.5%, 7/155, Table 1), two females (2.5%, 2/79), and five males (6.6%, 5/76), with no statistically significant difference between the sexes ($X^2: 1.47$, $df=1$, $p= 0.225$). Prevalence on the left foot is 2.8% (4/143, Table 2), 2.7% (2/73) in females, and 2.9% (2/70) in males. On the right side, the total prevalence is 2.7% (4/150, Table 3), with all four cases belonging to males (5.5%, 4/73). There is no association between laterality and the presence of the ossicle ($X^2: 2.07$, $df=1$, $p= 0.151$). The ossicle occurs bilaterally in one male (14.3%, 1/7), with unilateral cases prevailing, three in the left foot and three in the right foot. In five cases, the ossicle is fused to the medial cuneiform (Figure 8), and in two cases to the second metatarsal (Figure 9).

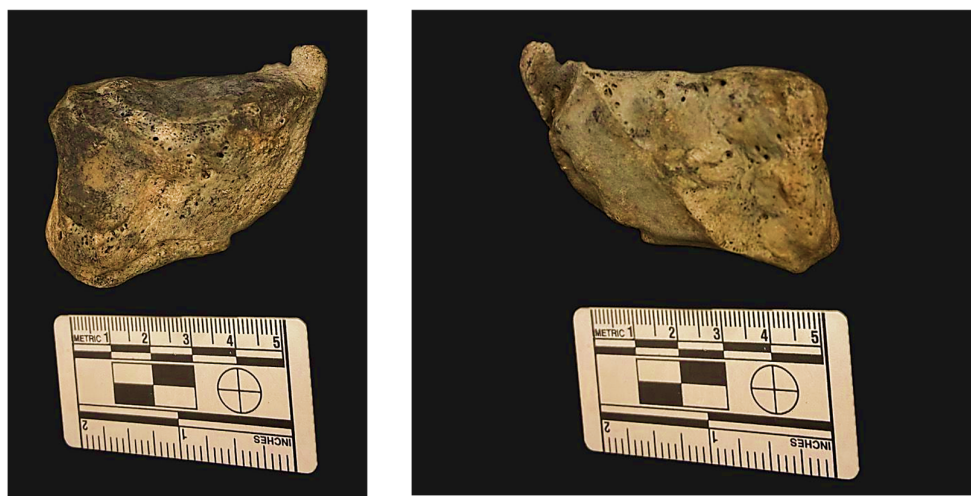


Figure 8. Distal and proximal view of the left medial cuneiform of a male individual (CISC/XXI/14) with a fused *os intermetatarsium*.



Figure 9. Proximal view of the 2nd right metatarsal of a male individual (CISC/XXI/58), showing the presence of a fused *os intermetatarsaleum*.

4. Discussion

This study reports a 21.4% prevalence (35/163) of accessory tarsal ossicles in a reference sample of Portuguese skeletal remains, a frequency broadly consistent with, though slightly higher than, that observed in a chronologically earlier Portuguese sample ([3]: 18.3%; 89/486). Seemingly, there were no changes over a relatively short period of time in the frequency of accessory tarsal ossicles in the Portuguese population – a particular interesting feature when accounting for the changing political and socioeconomic circumstances of the country [26]. The prevalence range reported in the broader literature spans from 18.0% to 40.2% [12,14,27–29], reflecting significant morphological variability across populations and diverse methodological approaches.

Non-anatomical factors may account for the relatively low frequency observed in our study, particularly the application of different study methodologies and samples [3], and the observation of distinct types of ossicles [30], including those associated with the tibia and fibula. Variation in the preservation and completeness of foot bones within the CISC/XXI may have constrained the identification of smaller ossicles. In addition, taphonomic alterations and the lack of specialized recovery procedures during the exhumation of the individual that compose the reference skeletal collection [25] could have further contributed to underrepresentation of these small and fragile bone variants.

The results corroborate previous findings that accessory ossicles are distributed similarly across sexes, with no statistically significant differences observed between males (27.5%) and females (15.7%)—a pattern consistently noted in other studies [3,12,14,28,31]. The co-occurrence of accessory foot bones was not observed. In fact, co-occurrence is not frequent [3,16], varying from 1.6% to 10.6% [3,12,14,28,31]. Usually, age is not considered as a demographic factor in the appearance or expression of foot ossicles and, as in this study, it is similar in both affected and non-affected individuals [3].

These findings reinforce the importance of accounting for anatomical variations in both clinical and anthropological contexts. Accessory ossicles, particularly in the tarsus, may mimic pathological conditions on radiographic imaging or influence biomechanical interpretations in bioarcheological reconstructions.

Os trigonum

Os trigonum is one of the most thoroughly documented in both anthropological and clinical literature [1–3,12,14,31]. Cerrato et al. [32] noted that individuals engaged in activities involving repeated plantar flexion (including, e.g., dancers [33]) are more likely to develop or retain this ossicle, suggesting a functional or biomechanical component in its manifestation. Studies based on medical imaging consistently report higher prevalence rates when compared to direct skeletal analyses. In the present sample, 15 (9.3%) individuals presented with *os trigonum*. Previous research studies report prevalence rates between 0.8% and 25.0%, varying by population and employed methodology [2,3,10,14,16,20,27–29,34–36].

The observed prevalence in the CISC/XXI skeletal sample supports the empirical hypothesis that medical imaging-based studies can overestimate the presence of the *os trigonum*, possibly due to its cartilaginous or partially ossified form in living individuals, which may not always persist or be recovered in dry bone collections. As previously noted, comparisons across studies require particular caution, as data collection methods vary considerably [3]. Most radiographic studies classify only unfused *os trigonum* as present, whereas osteological studies involving dissection or dry bone analysis can include both unfused and fused forms. The interpretation of fused cases is not always straightforward – particularly in distinguishing an enlarged posterolateral process of the talus from a completely fused *os trigonum* in the absence of a visible fusion line [37]. To avoid misclassification in this study, only cases with a clearly identifiable fusion line or sulcus were recorded as positive findings.

Regarding laterality, both the present study and previous literature suggest that *os trigonum* is predominantly unilateral, but without a noticeable propensity for the left or right foot. Bilaterality appears to be less common, with most studies estimating bilateral occurrence in only 5–10% of cases [3,12,14,16]. As with other accessory tarsal ossicles, no significant sex differences were observed in the present sample, a finding consistent with previous studies [3,12].

Calcaneum Secundarium

The prevalence of the *calcaneum secundarium* varies widely in the literature, ranging from 0.1% to 11.8% [3,14,35,36,38]. This variability can be attributed to differences in diagnostic methods, study populations, and inclusion criteria. In the present osteological sample, individual prevalence was 6.9%. Silva [36] reported a prevalence of 11.8% in a prehistoric Portuguese sample, significantly higher than in other populations such as four non-industrial groups (4.4%) and an early 20th-century U.S. sample (1.9%) [39]. Population homogeneity due to geographic isolation and/or marriage customs may account for the elevated rate in the prehistoric sample [36].

The relative uncommonness of CS partly explains the lack of robust data regarding laterality and sex distribution. In this study, CS was observed in three females (3.8%) and eight males (10.1%), with no statistically significant sex difference. Only three individuals – one female and two males – presented bilateral CS (27.3%, 3/11). A statistically significant association was found between CS presence and laterality, with a higher frequency on the right foot. However, earlier studies, such as Pfitzner's [10], described a tendency for bilateral occurrence, suggesting potential population-specific patterns.

The most common location for CS was the anterior facet of the calcaneus, specifically on the anteromedial aspect. Nine calcanei with CS had continuous facets, while five showed separate ones. Anderson [17] associated CS with separated facets, whereas Mann [39] found it more commonly associated with continuous facets. Future studies could clarify this relationship and offer further insights into the interaction between facet morphology and the presence of CS.

Os Sustentaculum

Os sustentaculum is generally asymptomatic and clinically insignificant but, in certain cases, it can become symptomatic [2]. This accessory ossicle was not observed in the sample from the

CISC/XXI, aligning with its rarity, as reported prevalences fluctuate between 0% and 1.0% [1,10,28,30,40]. The first documented instance of this ossicle was by Pfitzner in 1896, who recorded a frequency of 0.5% in dissected feet [10].

Os Vesalianum

Os vesalianum is a rare ossicle located at the proximal base of the fifth metatarsal within the *peroneus brevis* tendon [3,41]. It was first identified by Andreas Vesalius in the *De Humani Corporis Fabrica* [3], and later studied by Pfitzner [10] and Dwight [15]. Its prevalence ranges from 0% to 5.9% [3,10,14,27–29,42]. In this study, the ossicle was identified in a single female individual (1.3%) on the right foot.

This ossicle is typically round or oval and can articulate with the proximal end of the fifth metatarsal. In the present case, the ossicle showed well-defined cortical margins. Some studies suggest that it is slightly more common in males [28,42].

Accessory Navicular

The accessory navicular – also referred to as *os tibiale externum* or *naviculare secundarium* – is located medially to the navicular tuberosity [1]. It is one of the most common accessory foot bone in clinical settings and is usually asymptomatic. When symptomatic, it often manifests during childhood or early adulthood as localized medial foot pain [43]. Prevalence ranges from 1.2% to 28.3% in medical studies [10,14,16,27,38,44–46]. In anatomical and archaeological collections, however, lower prevalence rates are consistently observed, varying from 1.0% to 5.0% [3,47,48]. In the present study, this ossicle was identified in only one male individual (0.6%), located on the right foot. The ossicle corresponded to a Type II accessory navicular – the most common of the three types and the most easily recognizable in human skeletal remains [47].

Some studies convey a higher frequency in females [43,49,50], while others report no significant differences between sexes [3,44,47]. Similarly, reported patterns of lateralization differ across studies: while some authors describe a bilateral predominance [10,49,51], others have found no consistent asymmetry [44,45].

Os Intermetatarseum

This anatomical variant is uncommon, with estimated prevalence rates ranging from 0.0% to 12.5% [3,10,12,15,27,28,46,52,53]. In the CISC/XXI sample, the prevalence of *os intermetatarseum* is 4.5 %, consistent with previous studies but higher than in another Portuguese skeletal collection [3]. Bilaterality was only observed in a male individual (14.3%), below the rate reported by Case et al. [53]. Usually, anatomical / anthropological studies convey higher prevalences than medical imaging studies [3]. The discrepancy may stem from the ossicle's small size and frequent fusion, as skeletal analyses allow for direct inspection and clearer identification of fused variants, especially those involving the 1st and 2nd metatarsals [53].

5. Conclusions

The identification and documentation of accessory foot ossicles in reference skeletal collections are essential for advancing both anthropological and clinical knowledge of foot anatomy. The present study, based on the 21st Century Identified Skeletal Collection, provides updated data on the prevalence and distribution of these anatomical variants in a modern Portuguese sample. The results show a relatively low prevalence of these ossicles that is similar to a chronologically older sample from Portugal. Limitations of this study include potential underrepresentation due to preservation variability, taphonomic loss, and differences in recording methods. Despite these constraints, this research highlights the relevance of studying accessory foot ossicles in reference skeletal collections as sustained interdisciplinary investigations (integrating skeletal, clinical, and imaging approaches)

can advance the scientific understanding of these anatomical variants and their effects on human variation and health.

Author Contributions: Conceptualization, F.C. and A.M.S.; methodology, L.S., F.C. and A.M.S.; validation, F.C. and A.M.S.; formal analysis, L.S.; data curation, L.S.; writing—original draft preparation, L.S., F.C. and A.M.S.; writing—review and editing, L.S., F.C. and A.M.S.; supervision, F.C. and A.M.S. All authors have read and agreed to the published version of the manuscript.

Funding: The co-authors Francisco Curate and Ana Maria Silva were financed by the R&D Unit Research Centre for Anthropology and Health, with the reference UIDB/00283/2020, funded by the Foundation for Science and Technology (FCT/MCTES) through national funds (PIDDAC) (<https://doi.org/10.54499/UIDB/00283/2020>).

Institutional Review Board Statement: Research clearance was granted by the curator and the Scientific Commission of the Laboratory of Forensic Anthropology (21st Century Identified Skeletal Collection).

Informed Consent Statement: Human skeletal remains prevail as a material embodiment of once-living people but informed consent is not an issue since the individuals included in the study are long deceased.

Data Availability Statement: Data generated during this study are available upon reasonable request.

Acknowledgments: We are indebted to the curator of the CISC/XXI, Professor Maria Teresa Ferreira.

Conflicts of Interest: The authors declare no conflicts of interest.

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