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**Article**

# Variability of lesions and lesion patterns in possible treponematosis cases: Insights from an archaeological site in Tomar, Portugal

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With 8 figures and 2 tables

**Abstract:** *Objective:* This study aims to describe various types of skeletal lesions and lesion patterns observed in five potential cases of treponematosis from the same archaeological site. *Material and methods:* The study examines five adult skeletons recovered from Santa Maria do Olival, Tomar, Portugal. Macroscopic observations were conducted on all remains, supplemented by relevant radiographic investigations. *Results:* The analyzed individuals exhibited diverse types of skeletal lesions and distinct patterns of lesion distribution. Radiocarbon dating of two of these skeletons locates them chronologically close to the beginning of the colonial period (15$^{th}$ century). *Discussion:* The findings indicate considerable variation in the lesions and lesion patterns associated with treponematosis, even within the same archaeological site. The radiocarbon data implies the presence of pre-Columbian treponemal disease or a rapid progression to the tertiary phase of the disease. This study represents the oldest cases of treponematosis in Portugal and the only one with more than one individual affected from the same archaeological site. Considering Portugal's prominent role in the European Age of Exploration, the study underscores the significance of pathogen exchanges between Europeans, South Americans, Africans, and Asians. Future investigations should reassess Iberian osteological collections from this time period, given the historical importance of Portugal and Spain in the intercontinental movements.

**Keywords:** Paleopathology; Syphilis; Infectious diseases; Medieval/Post-Medieval; Caries sicca

## 1 Introduction

Treponematoses are usually divided into four groups: syphilis, endemic syphilis (or bejel), pinta and yaws. Their origins, in particular syphilis, are still the most discussed mystery in paleopathology, despite the efforts from historical (Quétel 1990), paleopathological (e.g. Baker & Armelagos 1988; Harper et al. 2011; Walker et al. 2015) and genetic research (Šmajs et al. 2012; Majander et al. 2020). Despite being thought of as a disease of the past, syphilis re-emerged in several regions (e.g. Stamm 2016) even in its congenital form (Cooper et al. 2018). The World Health Organization (WHO 2021) estimated that about 7.1 million new cases of syphilis occur globally in 2020.

Syphilis is a sexually transmitted infection with ubiquitous distribution, and can also be passed from an infected pregnant to the fetus via the placenta resulting in congenital syphilis (Ortner 2003). Endemic syphilis, on the other hand, is associated with dry and arid environments and acquired orally, where there are usually the first lesions, alongside the nasal and pharyngeal mucosa (Farnsworth & Rosen 2006). It has been found mostly among indigenous populations in North Africa, the Near East and temperate Asia (Roberts & Buikstra 2019). Pinta is from tropical climates and usually does not affect the skeleton (Farnsworth & Rosen 2006). Yaws are also from humid tropical environments and can be acquired through wounds or direct skin-to-skin contact (Farnsworth & Rosen 2006). The differentiation between the different treponematoses in skeletons is difficult, if not impossible (Roberts 2000; Powell & Cook 2005) despite several attempts (e.g. Steinbock 1976; Rothschild & Rothschild 1995). Baker et al. (2020) even suggest that from the patho-


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physiological viewpoint, the group of treponematosis (syphilis, endemic syphilis and yaws) are the same disease, which is caused by the same species of pathogen, and their reported clinical differences are the result of various factors such as mode and route of infection, age of acquisition and variation in host response.

The genetic literature on treponematoses is complex. Gray et al. (2006) suggested that *Treponema pallidum* seems to have too much variability to have evolved in the last 500 years. According to Harper et al. (2008), the *T. pallidum* subspecies evolved from yaws in America even though non-venereal forms could have originated in Europe, but Mulligan et al. (2008) commented that this study could have some limitations. Šmajs et al. (2012) reviewed the literature on the genetic diversity of *T. pallidum* and argued that its subspecies are too similar, even when compared to rabbit Treponema species (Strouhal et al. 2007). The authors (Šmajs et al. 2012) did not find support for the Columbian (Crosby Jr. 1969) or the pre-Columbian hypothesis (Holcomb 1934, 1935; Hackett 1963, 1967) but instead, an origin from the Treponema having the baboon as host. The work of Arora et al. (2016) suggests that a pandemic strain cluster that emerged in the mid-twentieth century would be the common ancestor of modern syphilis. In a recent review, Baker et al. (2020) propose that current genetic evidence allows the rejection of the Evolutionary hypothesis and that skeletal evidence allows the rejection of the Columbian hypothesis. Majander et al. (2020), found a variety of strains related to both syphilis and yaws in skeletons from the early modern period in Northern Europe, as well as a previously unknown *T. pallidum* lineage. This suggests the presence of treponematosis (both syphilis and yaws) in Europe, before the first recorded epidemic events at the end of the 15$^{th}$ century (Majander et al. 2020) even if not as common. Walker et al. (2015) studied 25 skeletons with treponemal disease from the medieval burial ground of St. Mary Spital, in London, and observed a clear increase in the prevalence of the disease in the 15$^{th}$ century.

In Portugal, all the possible cases of treponematosis are from the 15$^{th}$ to 18$^{th}$ centuries (Codinha 2002; Santos 2004; de Souza et al. 2006; Lopes 2008; Ferreira et al. 2013; Assis et al. 2015; Rosa et al. 2018) and are summarized in Table 1. From collections of identified skeletons, there are 57 individuals diagnosed with syphilis in the Collection of Identified

**Table 1.** Summary of the Portuguese archaeological possible cases of treponematosis and the affected bones (sk: skeleton).

| Site | Chronology | Collection size | Number of cases | Sex | Age at death | Affected bones | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Skull | Post-cranial skeleton | |
| Monasteryof Santa Maria do Pombeiro, Felgueiras | 12$^{th}$–19$^{th}$ | 22 | 2 | Undetermined | Adult | 1 fragment with caries sicca | 1$^{st}$sk: 2 tibiae and left femur with periosteal and gumatous lesions. 2$^{nd}$sk: Bilateral and symetric periosteal lesions on the diaphysis of long bones. | Lopes 2008 |
| Vale da Gafaria, Lagos | 15$^{th}$–17$^{th}$ | 11 | 1 | Male | 30–40 yo | Remodeled bone on the nasal area | Lytic lesions on the right hand, sternum and right clavicle | Ferreira et al. 2013 |
| Church of the Convent of Carmo, Lisbon | 16$^{th}$–18$^{th}$ | 150 | 2 | 1 Male; 1 Female | Adults | Caries sicca | Periosteal lesions on the lower limbs | Codinha 2002 |
| Royal Hospital of All-Saints, Lisbon | 18$^{th}$ | 14 | 1 | Female | Adult | Caries sicca | Bilateral new bone formation on the limbs | Assis et al. 2015 0 |
| Church of Sacramento, Lisbon | 18$^{th}$ | ? | 1 | Female | 18 months | Porotic areas on the parietals | Periosteal lesions on the limbs | de Souza et al. 2006 |
| Ermida do Espírito Santo, Almada | 18$^{th}$ | 88 | 1 | Female | 30–49 yo | Caries sicca | Diaphysis thickening of the right humerus and femur and the left clavicle and tibia | Rosa et al. 2018 |
| Largo de Palhães, Ribeira de Santarém | 18$^{th}$–19$^{th}$ | ? | 1 | Male | Young Adult | Caries sicca | Multifocal lytic lesions with periosteal lesions on the tibiae and right humerus and radius | Santos 2004 |

skeletons from the University of Coimbra (19th/20th centuries; Lopes et al. 2010; Lopes 2014; Lopes 2019). Out of these 57 identified skeletons none had lesions compatible *with caries sicca*.

In this study, we describe five possible cases of treponemal disease at different healing stages and with different lesion distribution patterns. This study builds on previous research to establish the distribution and variability of lesions and patterns of treponemal diseases in past populations.

## 2 Material and methods

### 2.1 Sample

The five skeletons described in this study were recovered from Santa Maria do Olival burial ground (12th to 18th centuries AD) in Tomar, central Portugal. These individuals were found in different areas of the graveyard and buried in the same Christian manner as the other members of the community. From the area intervened, approximately 6.500m$^2$, were recovered 3.675 primary inhumations and 1,456 ossuaries in a total of 6,792 minimum number of individuals (4,991 adults and 1,801 non-adults). However, not all the skeletons have been minutely studied so far.

Tomar was a Templar town with a very important military role in consolidating the Kingdom of Portugal by resisting the advances of the last Moroccan king of Hispania, Iacub ben Iuçuf Almançor (França 1994). The construction of the Convent of Christ, a Templar stronghold, started in 1160. It was most likely about this time that the Church of Santa Maria do Olival was constructed (Conde 1996) and became the pantheon of the Grand Masters of the Temple. However, the distribution of the skeletons around the church, of all ages and both sexes, suggests that Santa Maria do Olival collection represents the general population of Tomar and not only the individuals from the religious military orders (Curto et al. 2019).

The diet at Tomar was complex, low in terrestrial animal protein and high in aquatic protein intake, despite its inland location (Curto et al. 2018; Curto 2019). This diet rich in aquatic protein was discussed as a possible result of dietary restrictions imposed by the religious military order. Stable isotope data suggest no dietary differences between sexes (Curto et al. 2018; Curto 2019). The diet of two (SMOL 16.225 and SMOL 18.158), out of the five individuals studied, was previously estimated and compared with the estimated diet of the general population, suggesting similar diets (Curto et al. 2019).

### 2.2 Methods

Sex was estimated based on pelvic and cranial features, following Buikstra and Ubelaker's suggestions (1994). Adult age-at-death estimates employed a combination of skeleton maturation (Scheuer & Black 2004), pubic symphysis degeneration (Brooks & Suchey 1990; Buikstra & Ubelaker 1994), and auricular surface degeneration (Lovejoy et al. 1985).

A comprehensive analysis was conducted on the skeletons using both macroscopic and radiographic observations. The observations were carried out using the digital system MammoDiagnost UC Philips, with settings at 28 kV and 25 mA, utilizing Kodak Min-R screen film. The purpose of these observations was twofold: to describe the lesions present and to identify any additional anomalies that could assist in the process of making a differential diagnosis. Lesions were classified as consistent with treponemal infection but not pathognomonic, strongly suggestive of treponematosis, or pathognomonic according to Baker et al. 2020. In the presence of at least one tibia and another long bone or preserved cranium, the authors (Baker et al. 2020) consider the minimal conditions to observe the presence or absence of treponematosis are met.

## 3 Results

A summary of the type of lesions observed in each skeleton can be consulted in Table 2, while Fig. 1 illustrates the distribution of the lesions in the different individuals. All the individuals under study are adults and display lesions in both the cranial and postcranial skeleton.

### 3.1 Paleopathological lesions

#### 3.1.1 SMOL 14.22

This skeleton exhibits morphological features compatible with those of a young female adult, between 20 and 30 years old (Buikstra & Ubelaker 1994; Scheuer & Black 2004). The frontal bone has taphonomic alterations in the areas of the lesions, still, sclerotic diplöe can be observed near the metopic suture (Fig. 2) and two others, one on the right and another one on the left of the metopic suture. There is another lesion observed in a fragment of a parietal, presenting aggregate porosities. With the aid of a magnifying glass, we can observe that at least one of the lesions has raised, rounded margins. The endocranium also shows porosity and new bone growth. The cranium's fragmentation did not allow for measuring the dimensions of the lesions. The occipital and temporal bones do not present any macroscopic lesions. Zygomatic bones have porosity. The hard palate presents microporosity and a layer of new bone growth coating the right side. There are no dental caries or antemortem tooth loss that could be related to the changes on the hard palate. The mandible is thickened but without lesions on the bone's surface. Both the ribs and the vertebrae are poorly preserved, but the few fragments present do not present any lesions. The sternum body shows vascularisation, but it is not possible to observe the manubrium. The clavicles show generalised porosity and new bone growth, more severe in the sternal

**Table 2.** Summary of the type of lesions observed in each skeleton and their status (C: consistent with treponemal infection but not pathognomonic; ST: strongly suggestive; P: pathognomonic.). *Hackett maximum score; no – not observable; a – absent.

| | SMOL 14.22 | | SMOL 16.225 | | SMOL 18.158 | | SMOL 18.188 | | SMOL 20.240 | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Sex** | Female | | Male | | Male | | Female | | Male | |
| **Age** | 17–30 yo | | 25–34 yo | | 30–39 yo | | > 27 yo | | > 30 yo | |
| **Radiocarbon dating** | - | | - | | - | | 1458–1639 AD | | 1426–1515 AD | |
| **Element** | **Skeletal changes** | **Status** | **Skeletal changes** | **Status** | **Skeletal changes** | **Status** | **Skeletal changes** | **Status** | **Skeletal changes** | **Status** |
| **Nasal cavity** | Not observable | | Not observable | | Abnormal porosity | C | New bone formation | C | Not observable | |
| **Palate** | New bone formation | C | Not observable | | Abnormal porosity | C | Abnormal porosity, perforation | P | Not observable | |
| **Frontal*** | 4 | ST | Not observable | | 1 | ST | 5 | ST | 6 | P |
| **Parietal*** | 1 | ST | Not observable | | Without observable lesions | | 1 | ST | | |
| **Clavicle** | Porosity and bone formation | - | Local enlargements of bone; rugose surface, and focal superficial cavitation. Bilateral | | Longitudinal striae and new bone formation | C | Local enlargements of bone; rugose surface, and focal superficial cavitation. Bilateral. | P | Without observable lesions | |
| **Thoracic and lumbar vertebrae** | Without observable lesions | | Without observable lesions | | Without observable lesions | | Without observable lesions | | Without observable lesions | |
| **Articular surfaces of knee, ankle, hip, and elbow** | Without observable lesions | | Without observable lesions | | Without observable lesions | | Without observable lesions | | Without observable lesions | |
| **Hand and foot bones** | Without observable lesions | | Without observable lesions | | New bone growth | | Without observable lesions | | Without observable lesions | |
| **Humerus** | Not observable | | Local enlargements of bone; rugose surface, no bone destruction. Bilateral | ST | Local enlargements of bone; rugose surface, and focal superficial cavitation. Unilateral. | ST | Local enlargements of bone; rugose surface, and focal superficial cavitation. Unilateral. | ST | Without observable lesions | |
| **Radius** | Not observable | | Local enlargements of bone; rugose surface, and focal superficial cavitation. Bilateral. | P | Local enlargements of bone; rugose surface, and focal superficial cavitation. Bilateral. | P | Without observable lesions | | Without observable lesions | |

**Table 2.** continued.

| Element | Skeletal changes | Status | Skeletal changes | Status | Skeletal changes | Status | Skeletal changes | Status | Skeletal changes | Status |
|---|---|---|---|---|---|---|---|---|---|---|
| **Ulna** | Not observable | | Local enlargements of bone; rugose surface, and focal superficial cavitation. Bilateral. | P | Local enlargements of bone; rugose surface, and focal superficial cavitation. Bilateral. | P | Without observable lesions | | Without observable lesions | |
| **Femur** | Local enlargements of bone; rugose surface, and focal superficial cavitation | ST | Local enlargements of bone; rugose surface, and focal superficial cavitation. Bilateral. | P | Local enlargements of bone; rugose surface, no bone destruction. Bilateral | ST | Longitudinal striae and new bone formation | C | Local enlargements of bone; rugose surface, and focal superficial cavitation. Bilateral. | P |
| **Tibia** | Longitudinal striae and new bone formation | C | Local enlargements of bone; rugose surface, and focal superficial cavitation. Bilateral. | P | Local enlargements of bone; rugose surface, and focal superficial cavitation. Bilateral. | P | Without observable lesions | | Without observable lesions | |
| **Fibula** | Longitudinal striae and new bone formation | C | Local enlargements of bone; rugose surface, and focal superficial cavitation. Bilateral. | P | Local enlargements of bone; rugose surface, and focal superficial cavitation. Bilateral. | P | Without observable lesions | | Without observable lesions | |
| **Acquired treponemal disease scoring** (Harper et al. 2011) | 5 | | | | 2 | | | | 5 | |

extremity and inferior surface of the bone. It is not possible to analyse the scapulae, as they were poorly preserved. On the right side, the distal extremity of the humerus and the proximal extremity of the radius and ulna show some alteration of the bone surface. These lesions may result from bone growth, but the extensive taphonomic changes difficulted the bone surface observation. It was not possible to analyse the left humerus, radius, and ulna. Hand bones without lesions. All the diaphysis of the right femur shows striation, but only the distal third of the diaphysis has changes in the morphology and bone dimensions, resulting from the depositions of bone on the posterior side. The left femur is too fragmented to allow an examination of the bone. The right tibia presents striation mostly on the distal portion of the diaphysis and new bone growth around the malleolus. The right fibula only has striations along the diaphysis indicating healed periosteal reaction. From the left tibia, it was only possible to observe a few fragments, which had a thin layer of new bone. The

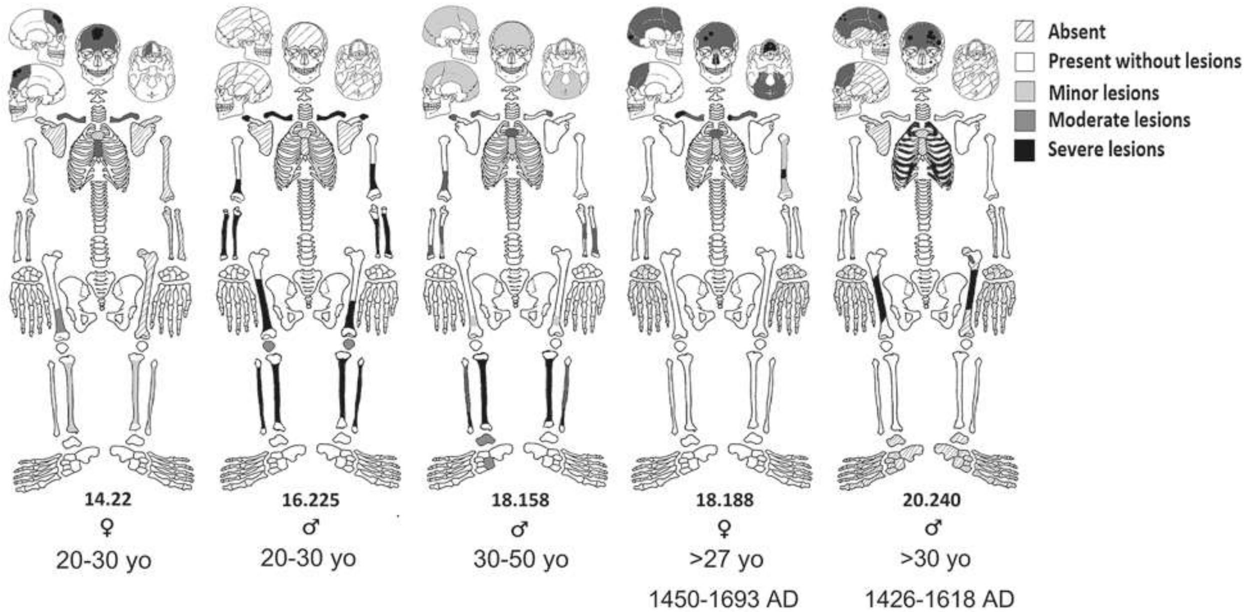


**Fig. 1.** Distribution of the lesions in each skeleton, as well as bones absent and those present but without lesions. Minor lesion – porosity; Moderate lesions – bone growth/destruction; Severe lesions – osteoperiostitis and/or osteomyelitis.

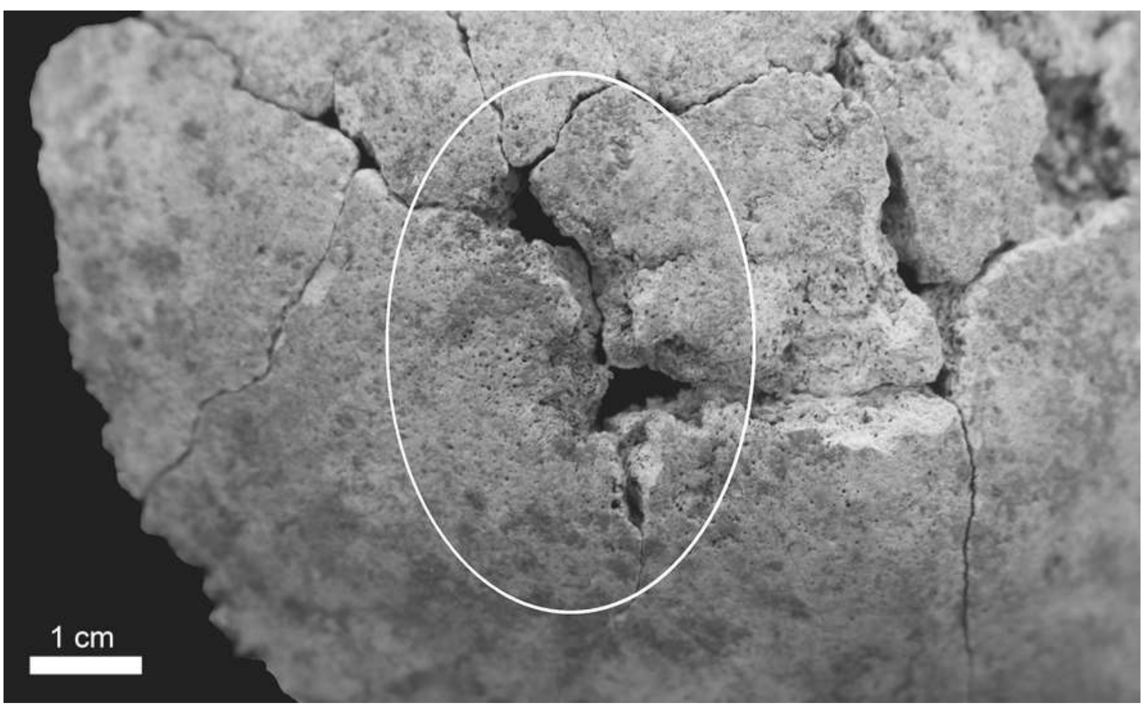


**Fig. 2.** Frontal fragment skeleton SMOL 14.22 showing a lithic lesion and porosity.

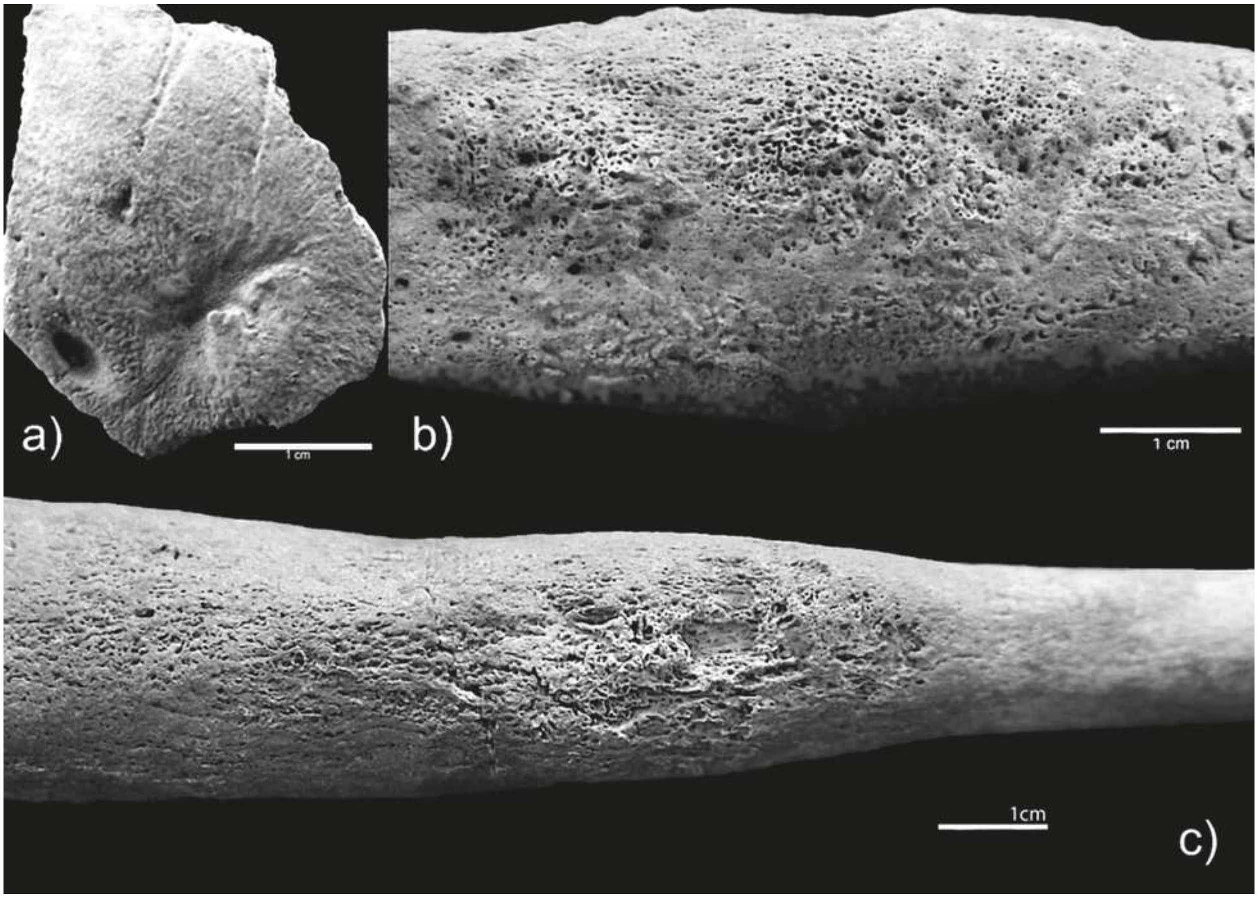


**Fig. 3.** SMOL 16.225; **a)** Lesion with a radial aspect on a right parietal fragment; **b)** Thickened left radius with bone growth and irregular surface and shape; **c)** Ulna showing bone growth, perforating lesion surrounded by periosteal build-up and fusiform shape.

available fragments from the left fibula only have porosity. Foot bones do not have lesions.

### 3.1.2 SMOL 16.225

This individual was estimated as being a young adult male (Brooks & Suchey 1990; Buikstra & Ubelaker 1994). This skeleton has bilateral lesions in various bones (clavicles, humeri, radii, ulnae, femora, tibiae, and fibulae).

The right parietal of this skeleton has a lesion (Fig. 3a) between the lambdoid and sagittal sutures. This lesion has a radial remodelled aspect, but it is very small, and this is the only cranium fragment available and it was not considered for the diagnosis. The palate has bone growth and porosity without associated oral pathologies.

A few fragments of left ribs show thickening and porosity, mostly on the outer surface, while the vertebrae do not have lesions. Both clavicles are thickened, with sclerotic round projections, diffuse periosteal bone and focal pitting. Despite both clavicles having similar lesions, the left one is more affected than the right. Both acromions are also thickened and have superficial cavitation. The humeri and radii are thickened, with a reduced medullary cavity, periostitis and sclerotic placking and dense irregular lesions (Fig. 3b). The ulnae show fusiform hyperostosis and snail-track pattern, with most of the diaphysis thickened, except for its distal third (Fig. 3c). Additionally, the right ulna also displays a penetrating defect surrounded by periosteal build-up. Hand bones without lesions. Both femora, tibiae, and fibulae are thickened and deformed, with a reduced medullary cavity, osteoperiostitis and sclerotic placking. The femora are affected mostly on the distal half of the diaphysis, and the right femur is more affected than the left femur. The first metatarsals show new bone growth and vascularization.

### 3.1.3 SMOL 18.158

This skeleton was estimated as a male (Phenice 1969; Buikstra & Ubelaker 1994) between 30 to 50 years old (Brooks & Suchey 1990). This individual has lesions in the cranium, sternum, ribs, vertebrae, clavicles, right acromion, all long bones (except the left humerus), and right talus and navicular. The cranium of this individual exhibits some lesions with a circular shape with a porotic appearance on

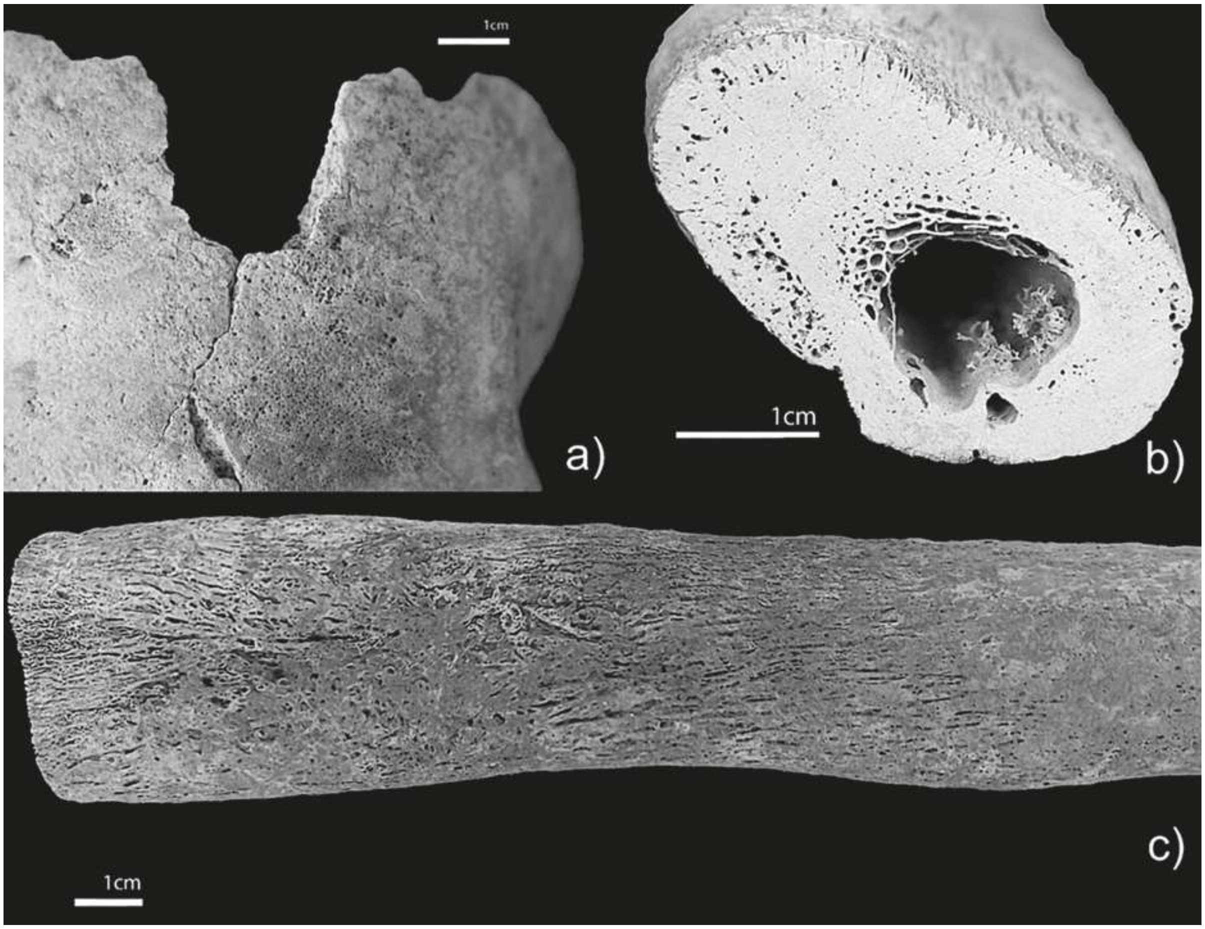


**Fig. 4.** SMOL 18.158; **a)** frontal bone showing porosity and circular lesions with porotic appearance; **b)** Transversal cut of the left tibia's diaphysis showing bone deposition; **c)** Left tibia with "snail-track" pattern.

the frontal (Fig. 4a). On the frontal bone, above the right orbit, there was a focus on porosity and another one above the left orbit. There is generalised porosity in the region of the sagittal suture, and above the sagittal suture, there is a sclerotic albescent lesion. Lesions were also registered on the endocranium with a serpentiform aspect and colour change in almost the total area of the frontal bone. Still, on the endocranium, there is an oval grey lesion with new bone formation. Porosity was also observed in the right zygomatic (the left bone was not present), palate, intermaxillary suture, and near the mandible foramina. The sphenoid has micro and macro porosity.

There were recovered fragments of at least 8 left and 10 right ribs. Among the left ribs there are at least 6 ribs with porosity and new bone growth on the visceral face but in different locations and at least one of these ribs had lesions on both faces of the rib. In the right ribs, it was possible to observe new bone growth in several fragments, mainly on the visceral side; at least 2 ribs have lesions on both faces. The fragmentation of these bones did not allow identifying which ribs had lesions. The manubrium shows new bone growth, porosity, and striation in both faces. The sternum body also shows porosity but had less severe lesions than the manubrium. Both clavicles have porosity and new bone growth near the acromial extremity. The right scapula presents new bone growth on the inferior side of the acromion, while the left scapula does not show any macroscopic changes. The distal third of the right humerus is thickened and irregular, with new bone growth. There are no observable lesions on the left humerus. Both radii present thickening on the distal third of the bones, new bone growth and porosity. The lesions are more severe in the left than in the right radius. The distal segment of the left radius shows obstruction of the medullary cavity, fusiform hyperostosis, and osteoperiostitis. The ulnae display bone thickening, fusiform hyperostosis, and osteoperiostitis. On the right ulna, the lesions are more severe than on the left one and more localized in the distal half of the bone. While on the left ulna, the lesions are visible mostly in the medial third of the extension. Bones from the hands do not show any macroscopic signs of lesions. The femora exhibit few changes, with only porosity and new bone growth in the distal end of the diaphysis. The tibiae (Fig. 4) have severe

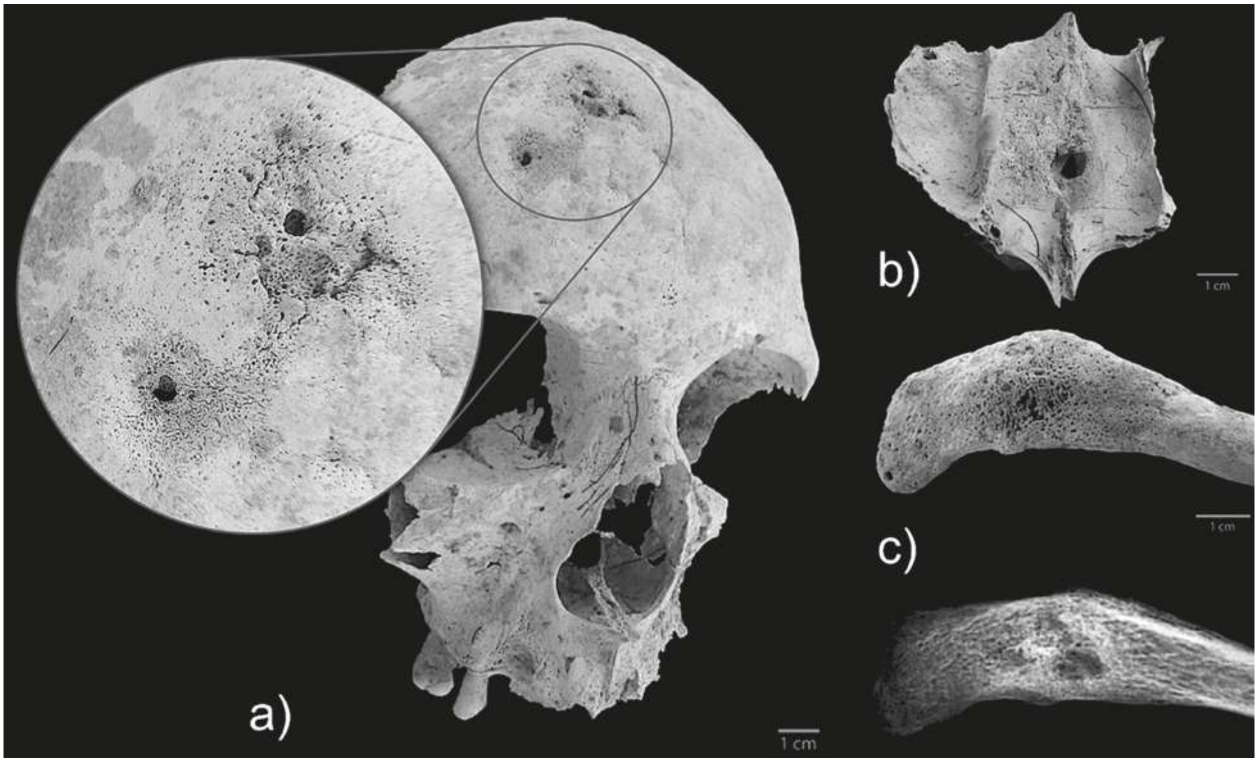


**Fig. 5.** SMOL 18.188; **a)** Frontal bone with destructive circular lesions and woven bone deposition; **b)** Palate's perforation and woven bone deposition; **c)** Perforating lesion surrounded by woven bone on the clavicle (photo and radiography).

lesions in all of the diaphysis: reduction of the medullary cavity, thickening resulting in shape loss, osteoperiostitis, "snail-track" pattern, very dense cortical bone, porosity and marked venous grooves. The fibulae show similar lesions to those observed in the tibia but are less severe and only in the distal end of the diaphysis. It was not possible to observe the right tibia and fibula. On the left foot, it was only observed light porosity on the talus. The right foot has lesions in the talus and navicular. In the inferior portion of the talus, thick bone deposition is surrounded by vascularisation and aligned with similar lesions on the navicular.

In addition to the lesions possibly related to treponematosis, this individual also has articular lesions in both femora and acetabula. Both femoral heads present a "mushroom" shape resulting from the extensive development of periarticular lipping, which is more evident in the right femur. The femoral heads seem to be in the correct anatomical position and without visible fracture lines. The acetabula also have lesions, particularly the right acetabulum, which are compatible with the lesions observed in the femoral head. The lesions observed in the foot might also be related to those on the femoral heads. These observations favour a diagnosis of Legg-Calvé-Perthes disease and not slipped femoral capital epiphysis as the last one shows inferior displacement of the femoral head, reducing the femur's length (Ortner 2003). There is another case of Legg-Calvé-Perthes disease in Tomar's collection (Curto & Fernandes 2011) also an adult male but with unilateral involvement. Bilateral involvement occurs in less than 20% of the cases (Ortner 2003).

#### 3.1.4 SMOL 18.188

It is estimated that this individual was a female (Phenice 1969; Buikstra & Ubelaker 1994) and over 27 years old (Scheuer & Black 2004). This individual had lesions on the skull, sternum, ribs, clavicles, left humerus and right femur (Fig. 1). On the frontal bone (Fig. 5a), between the orbits, there is porosity which in the centre is starting to agglomerate into a lesion. Right at the top, there are two small healed concave lesions and, on the left, a large depression, probably also a healed lesion as the bone is thickened in this area. Still on the frontal, towards the top, there are perforating lesions surrounded by a layer of woven bone and the largest lesion has raised rounded margins. These lesions can also be observed on the inner surface of the frontal, also surrounded by a layer of woven bone. Towards the back of the frontal bone, there is another depression, possibly a healed lesion. The right parietal of this individual, near the sagittal suture, presented a small area with agglomerated porosity, both on the exocranium and the endocranium. In the occipital, there is also a lesion with an agglomerate of porosity. The palate is perforated (Fig. 5b), with porosity and bone growth around the perforation. The mandible has a layer of new bone growth, on the right side, however, there are also several dental caries and antemortem teeth loss, therefore poor oral health may have also affected the mandible bone.

The sternum has bone growth with healed appearance and porosity. In a fragment of a right rib, in the external face, there was a circular lesion with microporosity. The vertebrae were fragmented but in the available fragments, no lesions were observed. The only bones in the upper limbs with lesions were the clavicles, the scapula, and the left humerus. The acromial extremity of the right clavicle has a perforating lesion with microporosity and new bone growth (Fig. 5c). The sternal extremity of the left clavicle is thickened and has destructive circular lesions, which partially destroyed the extremity of the bone. The acromion process of both scapulae also has porosity and bone growth. The left humerus has woven bone on the proximal extremity and an infectious focus with bone thickening in the distal extremity. The distal lesion has porosity and a mixture of compact and woven bone. In radiography, it is possible to observe a perforating lesion. In the lower limbs, only the right femur has slight new bone growth in the distal extremity. The tibiae and left femur are present, but have no lesions. The feet were not present when the skeleton was excavated.

#### 3.1.5 SMOL 20.240

This skeleton was estimated to be an adult male (Buikstra & Ubelaker 1994) over 30 years old (Scheuer & Black 2004). This individual had lesions in the skull, ribs, and left femur. The frontal bone has a confluence of several lesions (Fig. 6). A large lytic active lesion, between the coronal suture and the right orbit, that does not perforate into the endocranium. However, in the same region, there is some vascularization in the internal surface of the bone. Above the left orbit, there is a large lesion with raised rounded margins paired with vascularization on the internal surface of the bone. The right parietal is thickened, with a sclerotized look, and has at least two lesions. The left parietal is less affected than the right, but still has sclerotic thickened areas and a group of remodelled lesions. There are also remodelled lesions on the occipital, but it is still possible to observe the sunken look of the lesions with raised rounded margins. Above the left orbit, there are two lesions completely remodelled, only noticeable by the bone projection. The left zygomatic, below the infraorbital foramen, has a circular lesion, almost remodelled but still with bone projections, vascularization, and a thin top layer of bone.

The ribs of this individual are thickened with some alteration on the surface and shape of the bone (Fig. 7a). These lesions are completely healed into compact bone, without any porosity or woven bone. There are no observable lesions on the bones of the upper limb. The left femur is thickened (Fig. 7b), with loss of shape, reduced medullar cavity and surface changes that are completely healed, without signs of porosity or woven bone. The surface of the bones is irregular and has compact bone nodules and healed destructive foci. The tibiae do not have any pathological changes.

### 3.2 Radiocarbon dating and aDNA analysis

The radiocarbon dating was performed in 2019 on bone samples at the Beta Analytic Inc. Skeleton SMOL 18.188 (Beta – 513756) was dated with a 95.4% probability between 1458–1639 AD (54.1%: 1538–1639 cal AD; 41.3%: 1458–1530 cal AD) and with a 68.2% probability between 1450–1630 AD (38.8%: 1572–1630 cal AD; 29.4%: 1480–1522 cal AD). This skeleton was buried with a coin from the 13$^{th}$ to the 14$^{th}$ centuries. The individual identified as SMOL 20.240 (Beta – 516788) was dated with a 95.4% probability between 1426–1618 AD (87.9%: 1426–1515 AD; 7.5%: 1598–1618 cal AD), and with a 68.2% probability between 1438–1479 AD.

Ancient DNA analysis was carried out, for the five skeletons, at the Institute of Evolutionary Medicine, University of Zurich. Due to the low number of mapped reads, it was not possible to detect the ancient treponemal DNA.

## 4 Discussion

### 4.1 Differential diagnosis

For the differential diagnosis, we took into consideration conditions like Paget's disease of bone, mycotic infection, hypertrophic pulmonary osteoarthropathy, tuberculosis, leprosy, metastatic bone disease, and non-specific infections such as pyogenic osteomyelitis and periostitis. In Table 2, a summary of the lesions per individual and their respective statuses is provided (C: consistent with treponemal infection but not pathognomonic; ST: strongly suggestive; P: pathognomonic) according to Baker et al. 2020.

Paget's disease is characterised by cortical thickening, particularly of the skull (Waldron 2009), which was not observed in these individuals, despite the presence of cranial lesions. In the cases of Paget, one of the most frequent alterations is the bowing of bones, especially of the weight-bearing bones (Ortner 2003), which was not observed in the described skeletons.

Mycotic infections typically exhibit localized lesions in the area exposed to the fungi, rather than spreading extensively across the entire skeleton. These lesions predominantly involve the diplöe, distinguishing them from treponemal infections (Ortner 2003). In cases of systemic mycotic disease in the human skeleton, the lesions tend to be randomly distributed, displaying a more destructive nature (Grauer & Roberts 2019). However, the body's defence mechanisms can often contain the infection before it disseminates to the bone. Presently, fungal infections are exceedingly rare in Europe, and their occurrence in paleopathological records is even rarer, with only one potential case reported in Continental Europe (Curto & Fernandes 2016).


**Fig. 6.** Example of circular destructive lesions on the cranium of the skeleton SMOL 20.240.

Hypertrophic pulmonary osteoarthropathy usually has a bilateral manifestation on the joints, especially the wrist, ankle, and knee but without endosteal bone deposition and lesions on the cranium are rare (Aufderheide & Rodríguez-Martín 1998; Ortner 2003), which is not consistent with the lesions observed in these skeletons.

Tuberculosis affects the spine and ribs, with predominately lytic lesions (Ortner 2003). Comorbidity cannot be excluded as treponematosis does not frequently affect these bones (Aufderheide & Rodríguez-Martín 1998; Walker et al. 2015; Radu et al. 2016). In 1934, a Portuguese physician proved the relationship between syphilis and tuberculosis, stating that [the bacterium of] tuberculosis settles quickly and easily in lung syphilis lesions and its march in tissues weakened by syphilis is rapid and destructive (Carvalho 1934). Some cases of tuberculosis have already been diagnosed among the individuals exhumed from this necropolis (Silva 2020), so the co-existence between these two diseases, or between treponematosis and any other lung disease may have occurred.

Leprosy is characterized by the presence of rhinomaxillary syndrome and bone loss on the elements of the hands and feet (Waldron 2009), once again, these lesions are not compatible with those observed in the skeletons under study.


**Fig. 7.** SMOL 20.240; **a)** fragment from a thickened rib with an irregular surface; **b)** fragment from the femur, where it is possible to observe the thick compact bone deposition forming an irregular surface.

The lytic lesions observed in these individuals are more consistent with superficial cavitation without the pathognomonic cloaca associated with pyogenic osteomyelitis (Ortner 2003) and without the typical striation observed in periostitis (Ortner 2003). Still, the individuals might have other infections alongside the treponema. The skin ulcers would give easy access to viruses, bacteria, and fungi in the individual's bloodstream.

Bone metastatic disease tends to be more common among older individuals, and its occurrence in archaeological human remains is expected to be moderate due to demographic characteristics (Marques 2019). The lesions of the metastatic bone disease is more frequent in the axial skeleton and proximal areas of the appendicular skeleton (Rubens 1998). In the cranial vault, the most marked destruction is in the diploë, followed by the outer and inner tables (Marques 2019), opposite to the cranial lesions caused by treponemal infection (Hackett 1975, 1981).

The lesions observed, in particular, those in the skull, and the skeletons studied are compatible with the tertiary phase of treponema infections, as caries sicca is a pathognomonic indicator of this disease (Hackett 1975, 1981). Syphilis is the most probable cause for the lesions observed in these skeletons. However, an infection caused by other treponemas such as endemic syphilis and yaws cannot be excluded, particularly when its presence in Europe has been recorded in previous studies (Majander et al. 2020). Even though endemic syphilis is typical of hot deserted areas, it was also described as endemic in some European regions (Berco 2016) and pathologies not observed before in continental Europe have been coming to light (e.g. Cremasco et al. 2015; Curto & Fernandes 2016). Since Tomar was a Templar stronghold, it is also possible that the dissemination of the disease is related to returning from the Crusades. A rapid change in the transmission mode, from the skin to venereal contact, may have led to a transformation of the infec-

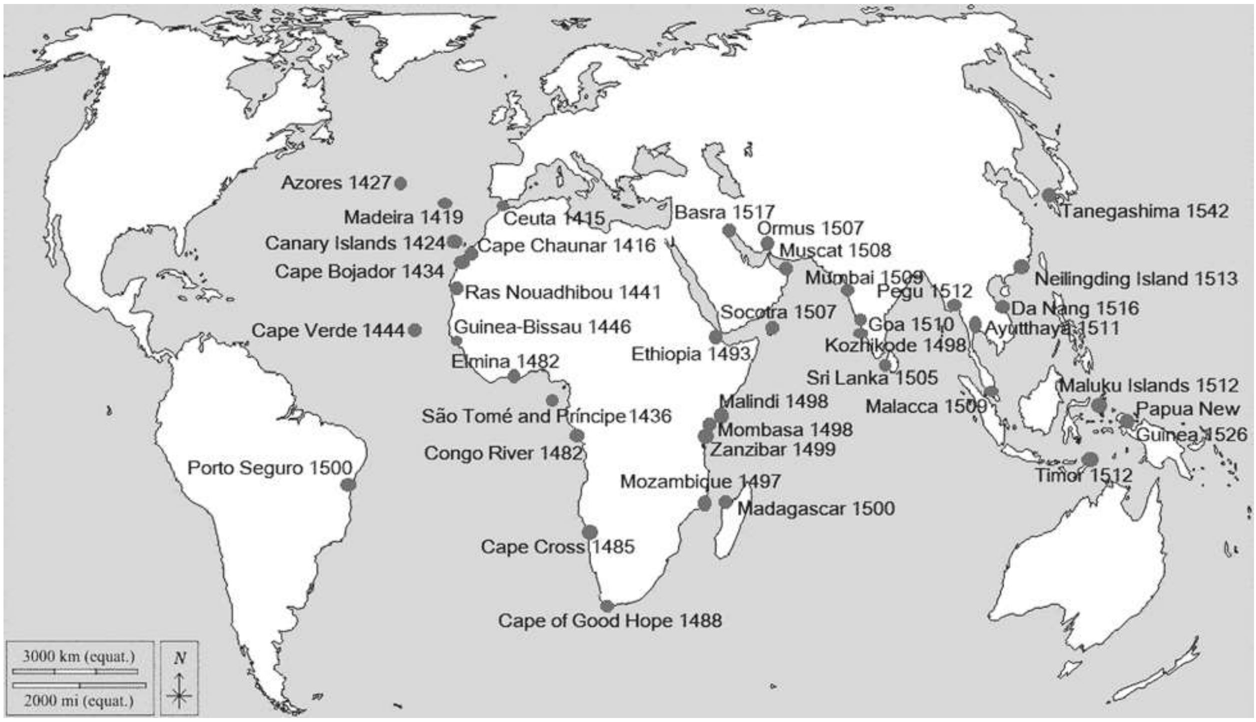


**Fig. 8.** Map of Portuguese maritime exploration with some of the first arrival places and dates. Main Portuguese spice trade routes in the Indian Ocean and territories claimed during the reign of King John III (c. 1536). Constructed based on Garcia 1992.

tion (Knell 2004). The radiocarbon dating of two of these skeletons (SMOL.20.240: 1426–1515 AD; SMOL.18.188: 1458–1639 AD) locates them chronologically close to the beginning of the colonial period, suggesting a pre-Columbian treponemal disease or a rapid progression of the disease to a tertiary phase. It is also important to note that, apart from South America, during the same chronological period (1415–1542), the Portuguese were also travelling to other parts of the world (around Africa to Asia) with different types of treponemas (Fig. 8). Yaws are endemic to American, African, and Asian tropical regions and Bejel is endemic to dry African and Asian regions (Farnsworth & Rosen 2006). Not only did the Portuguese start to move around the world at that time but also brought African slaves in 1441 (Morison 1940).

The difficulty of differentiating between the treponematoses in skeletal remains limits understanding of the pathogen's origin and evolution. The recent reconstructions of *T. pallidum* genomes from archaeological material (Schuenemann et al. 2018; Majander et al. 2020) have the potential to soon solve the syphilis enigma, but it is affected by DNA damage that begins immediately after death (Duchêne et al. 2020) and highly variable by regional geology, climate, weather, environment, mineral content, and exposure (Hansen et al. 2017).

## 4.2 Distribution of the pathologic lesions

It is generally agreed that there are bones more commonly affected by treponematosis, these being tibiae, nasal region and palate, and cranial vault (Steinbock 1976; Ortner 2003; Roberts & Buiskstra 2019). This high frequency of lesions in the tibia has also been registered in more recent studies (Lopes 2014; Walker et al. 2015; Radu et al. 2016) and some authors believe that it can be related to the association between the lymphatic and skeletal systems (Buckley & Dias 2002). Among the possible archaeological acquired cases of treponematosis in Portugal (Codinha 2002; Santos 2004; Lopes 2008; Ferreira et al. 2013; Assis et al. 2015; Rosa et al. 2018), all of the authors mention that the skeletons present *caries sicca* (Table 1), except the one from Lagos (Ferreira et al. 2013), and periosteal and/or lythic lesions on the long bones, more commonly in the lower limbs. The only cases with lesions in other bones but the skull and limbs are the one from 15th–17th century Lagos (Ferreira et al. 2013), who also presents lythic lesions on the sternum and clavicle and the one from 18th century Almada (Rosa et al. 2018) who also has lesions on the left clavicle.

In Tomar's collection, the bones more commonly affected with moderate to severe lesions were the frontal (4 out of 4 observable bones), the clavicles (4 out of 5 skeletons), and the tibiae and fibulae (3 out of 5 skeletons) (Fig. 1). These lesions are mostly bilateral and symmetric. All of these skeletons fit the expected descriptions of treponematosis, however, given the higher involvement of long bones on the skeleton 16.225, alongside his young age (25 to 34 years old), we cannot exclude the possibility of this one, in particular, being a case of yaws (Ortner 2003). Skeleton 20.240 is the only one in this group with severe lesions on the ribs, being all his lesions severe and completely remodelled. Since there is a 88% chance that SMOL 20.240 lived between 1426 and 1515 AD, it is also possible that the different pattern of the lesions in this skeleton might be related to a different lineage of treponematosis, present in Europe before the voyages of Columbus (e.g. see Majander et al. 2020). However, we cannot exclude the possibility of this individual being an example of the co-existence of treponematosis and tuberculosis.

This dissimilarity in lesion patterns observed in Tomar can also be a coincidence, given the small sample size. Previous studies observed continuity in the lesion's distribution between pre-Columbian and post-Columbian contexts in Britain (Walker et al. 2015) and treponematosis tends to have similar bone lesion patterns (Roberts 2000; Powell & Cook 2005). The five cases under study show lesions that vary in appearance, which can be related to their state of healing, the individual's immune response to the pathogen, the duration of active infection, and whether there is co-infection with other pathogens or even other treponemas (Mays 1998; Ortner 2003). Treatments with mercury can also influence the aspect of the lesions. Skeletons of people treated with mercury have been described as having more severe skeletal lesions than those who were not treated that way (Ioannou et al. 2015). The expression and severity of treponemal disease might have been more variable in the past, which hindrances diagnosis using modern criteria.

So far, no cases of congenital syphilis have been found in Tomar's collection, out of the 93 non-adults studied.

## 5 Conclusion

This study illustrates the possible variations of treponematosis lesions, both in the macroscopic aspect and distribution pattern. This variation, alongside the disease affecting the bone mostly in the tertiary phase, can explain the relatively few cases from Portugal published, despite the disease has affected so many people in the past. This is particularly true for skeletons with poorly preserved crania. This study builds on previous research to establish the distribution and variability of lesions and patterns of treponemal diseases in past populations. Our findings suggest a pre-Columbian treponemal disease or a rapid progression of the disease to a tertiary phase, as the radiocarbon dating of two of these skeletons (18.188:1458–1639 AD; 20.240:1426–1515 AD) locates them chronologically close to the beginning of Columbus voyages.

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