

Case study

Histology of pulmonary tuberculosis in a 19th-century mummy from Comiso (Sicily, Italy)

Raffaele Gaeta^{a,*}, Valentina Giuffra^a, Frank Maixner^b, Giacomo Aringhieri^c, Antonio Fornaciari^a

^a Division of Paleopathology, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy

^b Eurac Research Institute for Mummy Studies, Bolzano 39100, Italy

^c Academic Radiology, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy

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ABSTRACT

Objective: The aim of this study is to investigate potential evidence of tuberculosis in mummified remains.

Materials: The natural mummy of an anonymous friar from the mortuary chapel of the church of Santa Maria della Grazia in Comiso (Sicily)

Methods: The mummy was studied through macroscopic examination; tissue sampling was conducted through breaches in the dorsal surface of the thorax. Radiological, histological and immunohistochemical analyses were performed on the pulmonary parenchyma.

Results: The mummified remains are those of an adult male approximately 25–45 years old. In the left lung, 7 intra parenchymal calcified nodules were detected. The fibrocalcific nodules showed some lacunae surrounded by fibrous tissue containing amorphous necrotic, most probably caseous, material.

Conclusions: These findings are compatible with a chronic infectious-inflammatory disease, likely a calcification of a previous Ghon complex of an apical nodular tuberculosis.

Significance: Our study supports the great spread of the disease in the 19th century; a time when it reached its maximum peak in Europe.

Limitations: Molecular investigations failed to detect traces of *Mycobacterium tuberculosis* DNA in the sample.

Suggestions for further research: The investigation on the mummies from Comiso is still in progress, and further analyses will potentially provide paleopathological data on this community of Modern Age which could be integrated with historical and archival sources.

1. Introduction

Collections of mummies preserved in crypts and chapels are frequent in Italy, especially in the central and southern regions, and date back to the Middle Ages through the Renaissance to modern times. Sicily has the largest collections of mummies, exemplified by the bodies housed in the Catacombs of the Capuchin Friars in Palermo (16th–19th centuries) and the mummies of Savoca (18–19th century) (Fornaciari, 1998).

In Comiso (Sicily), the mortuary chapel called 'the Chapel of the Dead', annexed to the church of Santa Maria della Grazia, built by the Capuchin friars in the 18th century, contains 50 mummies lying in niches opened in the right and left lateral walls and above the entrance. A total of 107 skulls, some of which are mummified, are positioned on

oblique niches (Fornaciari and Gamba, 1993). All the individuals are males of different ages, belonging to the friars of the Capuchin Order (left wall) and to layman benefactors of the convent (right wall), who probably were greatly respected individuals of the local society of the time, so they received the honour of being buried in a privileged area. All the mummies wear Capuchin monastic clothes, most of which date back to the 18th–19th centuries; twenty mummies display a label with the name and date of death. The earliest reported date is 1742, while the most recent is 1838 (Amadei and Fornaciari, 1996). The mummies are natural, i.e. not subjected to evisceration or craniotomy. However, the bodies underwent a process of dehydration, favoured in southern Italy until the 19th century. This method consisted of draining the corpses' fluids in small rooms annexed to the chapel (Fornaciari and Gamba,

* Correspondence to: Division of Paleopathology, Department of Translational Research and New Technologies in Medicine and Surgery, Via Roma 57, Pisa 56126, Italy.

E-mail address: raffaele.gaeta@med.unipi.it (R. Gaeta).

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1993; Fornaciari et al., 2010; Fornaciari and Giuffra, 2006).

The series of mummies of Comiso is particularly interesting since it provides relevant information about the cultural practices and health status of a conventual monk and members of the secular Sicilian population of the 18th-19th century. Moreover, paleopathological investigations revealed several pathological conditions, such as atherosclerosis, a disease that we now know to have been present even in past populations (Gaeta et al., 2019), splenomegaly, acariasis, goiter (Ciranni et al. 1999), colon diverticulosis, inguinal-scrotal hernia, and varicose veins with ulcers of the lower limbs (Fornaciari and Gamba, 1993).

In this paper, we analyze the mummified remains of an adult male with calcified nodules investigated with radiological and histological techniques.

2. Materials and methods

During the survey performed by Professor Gino Fornaciari in 1987, all the 50 mummies were submitted to paleopathological investigations performed in a temporary laboratory set up on site, with detailed descriptions of their external appearance and state of preservation.

The natural mummy of an anonymous friar with no epigraph, labelled as Comiso 21 (CM21), dressed in a well-preserved Capuchin monastic cloth, and lying in a niche on the right wall near the entrance of the chapel, underwent tissue sampling. To avoid compromising the integrity of the body, the samples were taken through breaches already present in the posterior wall of the thorax, which exhibited large areas without skin and soft tissues (Fig. 1B). The present study is aimed at investigating the samples of individual CM21 through radiological, histological, immunohistochemical and molecular techniques.

Radiological investigations were not performed during the first survey, since it was not possible to move the mummies and no portable X-ray unit was available at the time. Later, however, a Computed Tomography (CT) scan of the lung samples was performed at the Pisa University Hospital using a third-generation scanner (GE Discovery RT; acquisition parameters: 80 kVp, 90 mA, 2,5 mm slice thickness, 36 cm DFOV).

The samples were processed according to the protocol established for mummified remains (Aufderheide, 2011). The pulmonary tissue was immersed for a maximum of seven days in Sandison's solution (Sandison, 1955). The calcified nodules were immersed in a biacid mixture for rapid decalcification (MicroDecfast, Diapath) for 24 hours.



Fig. 1. A. Individual CM21 with the well-preserved monastic dress of the Capuchin Order. B. The dorsal surface of the trunk shows a poor state of preservation. C. Two of the seven pulmonary calcified nodules.

The samples were then encased in a paraffin block, and histological slides approximately 3 μ m thick were obtained using a Leica RM2255 microtome and stained with classic haematoxylin and eosin (Fornaciari et al., 2019). For histochemical Ziehl-Neelsen staining, samples were stained with 3 % carbol fuchsin for 15 minutes, heated for 5 minutes and then cooled at room temperature. Stains were then washed with water, decolorized with 70 % Ethanol 1 % HCl and washed again with tap water. Slides were contrasted with 3 % methylene blue dye for 5 minutes, washed with tap water and left to dry (Atlas and Synder, 2011). For immunohistochemical staining, tissue sections (5 μ m) were deparaffinised, hydrated, and, after endogenous peroxidase inactivation, immunostained with BenchMark Ultra stainer (Ventana Medical Systems, Tucson, AZ), and revealed with VIEW DAB detection kit, providing a brown reaction product. The primary antibodies used were anti-Pan keratin (AE1/AE3/PCK26, Ventana Medical Systems, Tucson, AZ), anti-Cytokeratin 7 (SP52, rabbit monoclonal, Ventana Medical Systems, Tucson, AZ), and anti-Thyroid Transcription Factor-1 (8G7G3/1, mouse monoclonal, Ventana Medical Systems, Tucson, AZ). After completing the staining process, the slides were removed from the autostainer, counterstained with haematoxylin-eosin, dehydrated, and mounted with a permanent medium.

Both soft tissue (dorsal skin, left and right lung) and hard tissue (calcified nodules, coccygeal vertebra, rib) samples were subjected to DNA-based analysis as described by Maixner and colleagues (Maixner et al., 2023) with minor modifications. In brief, DNA was extracted from 50 to 100 mg sample using a linear polyacrylamide-based extraction technique (Maixner et al., 2022). DNA extracts were transformed in double-indexed library (Kapp et al., 2021) for the subsequent Illumina multiplex sequencing (HiSeqX, 2x150bp). Human DNA analysis and general bacterial profiling of the metagenomic shotgun dataset have been performed as described by Sarhan et al., 2023. The shotgun datasets were additionally screened for traces of *Mycobacterium tuberculosis* DNA using the bioinformatics pipeline described by Jäger and colleagues (Jäger et al., 2023).

3. Results

The body of individual CM21 is believed to be an adult male who died at 25–45 years of age based on the Suchey-Brooks method for age estimation from the pubic symphysis (Brooks and Suchey, 1990). The individual was in a good state of preservation, except for the head, which was missing due to post depositional events (Fig. 1 A). The skin and the soft tissues were still preserved in the upper limbs and in the anterior wall of the chest and abdomen, but only partially preserved in the lower limbs and the feet.

Macroscopic analysis revealed 7 intra-parenchymal calcified nodules with diameters varying between 2 and 7 mm (Fig. 1 C), which were sampled from the upper lobe of the left lung. Despite the quite low quality of the images due to the lung sample conditions, CT scan revealed a larger calcified intraparenchymal pulmonary nodule along with two calcified satellite smaller nodules (Fig. 2 A-C).

Histological study of the pulmonary parenchyma and calcified nodules showed numerous pathological alterations. Owing to the rehydration processes and to the natural fragility of the pulmonary tissue, the sample became partially fragmented which, though, did not compromise the widespread fibrosis or the intense deposits of dark pigments that could be associated with severe anthracosis (Fig. 3A). The tissue showed widespread fibrosis, and the alveoli appeared severely collapsed. We hypothesize that this is due to atelectasis, despite the compromised preservation of the tissue. In some samples there were small to medium sized circular structures characterized by a thick wall of fibrous tissue partially covered by residues of probable columnar epithelium compatible with bronchiolar structures (Fig. 3B). The fibrocalcific nodules showed some small lacunae surrounded by fibrous tissue containing amorphous necrotic tissue, similar to caseous material (Fig. 4A-B). However, histochemical Ziehl-Neelsen staining did not detect acid-fast bacilli due to preservation and thanatological events that resulted in the loss of bacterial structures. Immunohistochemical staining was inconclusive probably due to the decalcification process.

The DNA-based analysis revealed an overall poor endogenous DNA quality in the analysed soft and hard tissue samples. The endogenous

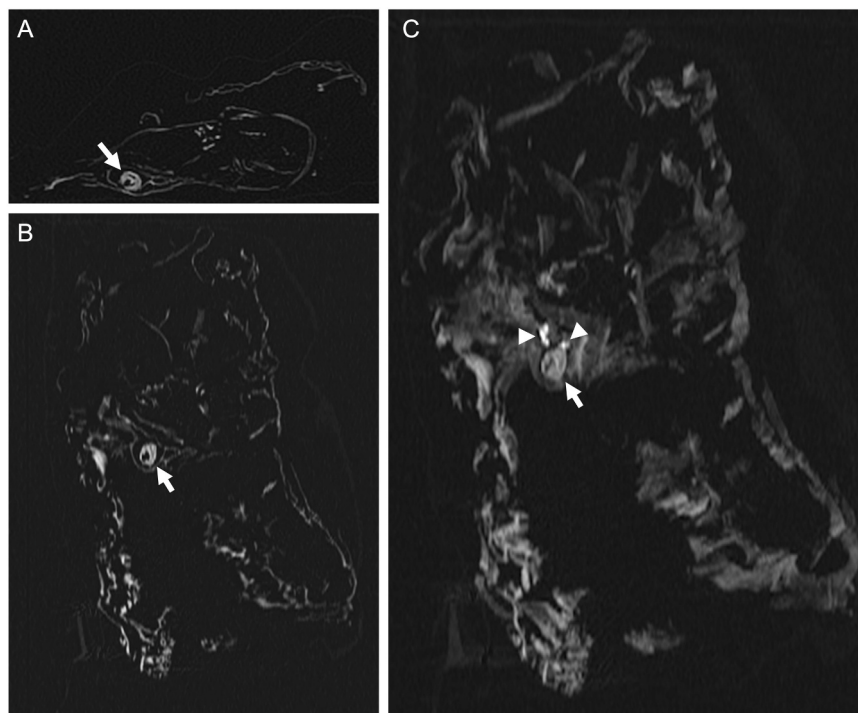


Fig. 2. CT scan of the lung samples showing the larger calcified intraparenchymal pulmonary nodule (arrow) and the two satellite smaller nodules (arrowheads). A: axial native image; B: reformatted coronal image; C: coronal maximum intensity projections with a 5 mm slice.

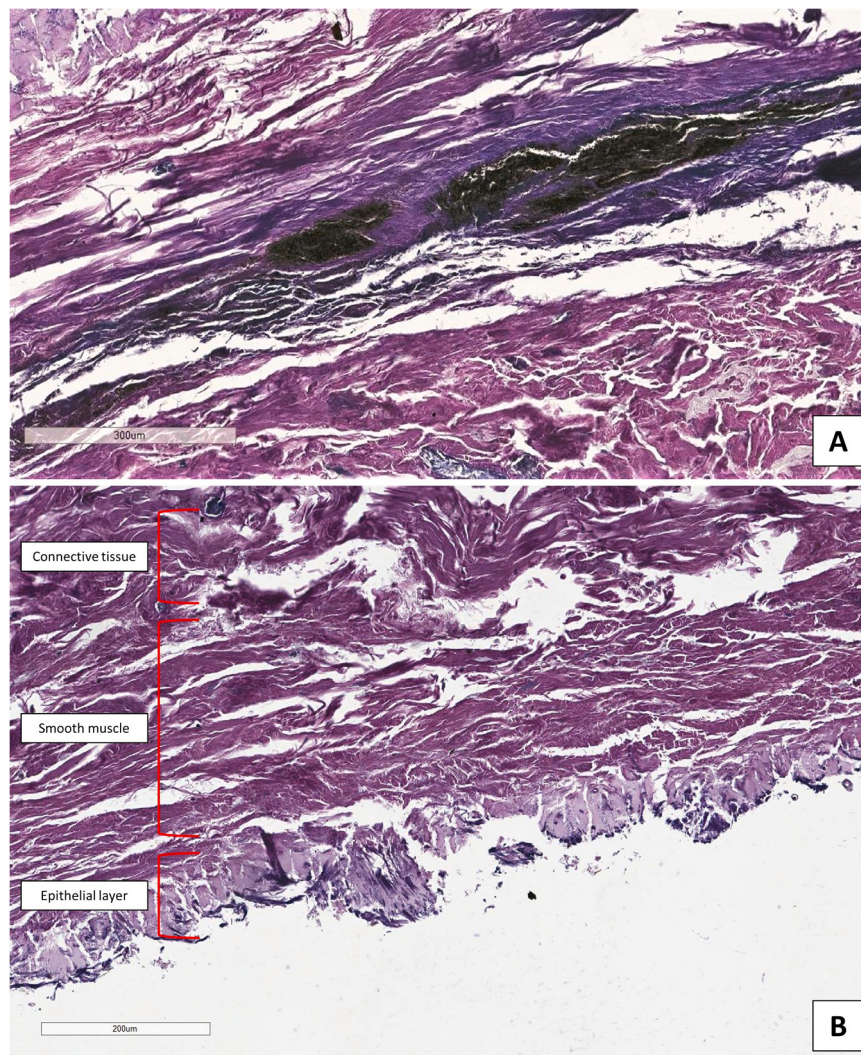


Fig. 3. A. Diffuse fibrosis of the pulmonary tissue with massive anthracosis (HE, 4X). B. A bronchiolar structure with residues of epithelial layer; smooth muscle layer and the surrounding connective tissue are also evident (HE, 10X).

human DNA content ranged from 0.04 % to 0.09 %. There were no traces of *Mycobacterium tuberculosis* DNA detectable. The majority of DNA could be assigned to different species of the genus *Clostridium* (e.g. *Clostridium putrefaciens*), which are members of the post-mortem bacterial community, indicating that the mummy was already in a state of decomposition.

4. Discussion and conclusions

Imaging examination and macro- and microscopic features of the calcified nodules require differential diagnosis. First, a primary malignant neoplasm of the lung can be excluded since calcifications in lung cancer are rare. When present, they typically exhibit amorphous or reticular patterns (Khan et al., 2010). The lesions could be attributed to a metastatic lesion; however, evidence of calcifications caused by primary cancer in organs other than the lungs were not observed. As for benign tumors, pulmonary hamartoma, i.e. the most common benign lung tumor, is composed of mature tissues that are normally present in the lung, but in disorganized growth; however, the typical appearance is that of a popcorn-ball calcification (Khan et al., 2010).

Sarcoidosis is a systemic disorder of unknown cause characterized by the presence of granulomas, typically non necrotizing. The typical feature is the galaxy sign, which comprises a pulmonary nodule created by the merging of numerous diminutive nodules that diverge from each

other as they radiate outward, resembling the appearance of a stellar galaxy (Nakatsu et al., 2002). Above all, histology in our case shows diffuse caseous necrosis, which is not observed in sarcoidosis lesions.

However, the findings observed in individual CM21 are more compatible with a chronic inflammatory, probably infectious disease, of the lungs, together with atelectasis, fibrosis and calcific nodes arising on a pulmonary parenchyma characterized by severe and widespread anthracosis and chronic pleuritic (Savic et al., 2022). Among inflammatory and infectious diseases, histoplasmosis, an infection caused by the fungus *Histoplasma capsulatum*, brucellosis, a bacterial disease caused by various *Brucella* species, and pneumoconiosis, a disease caused by inhalation of dust, should be evaluated (Marchiori et al., 2005). Calcified nodules from histoplasmosis are typically small and may be multiple, presenting as scattered, punctate calcifications throughout the lung parenchyma, presenting a pattern that may resemble "buckshot" or "eggshell" calcifications (Dylewski, 2011). These calcifications can also involve hilar and mediastinal lymph nodes, which may appear calcified (a "miliary" pattern). Calcifications in brucellosis are relatively uncommon in the lungs. When present, they tend to be less well-defined and can vary in size (Mirijello et al., 2023). The pattern of calcification in pneumoconiosis depends on the type of dust inhaled. In silicosis, for example, the nodules are typically small (1–5 mm), round, and can be calcified. These calcifications often have an "eggshell" appearance, particularly in the hilar lymph nodes. In coal worker's

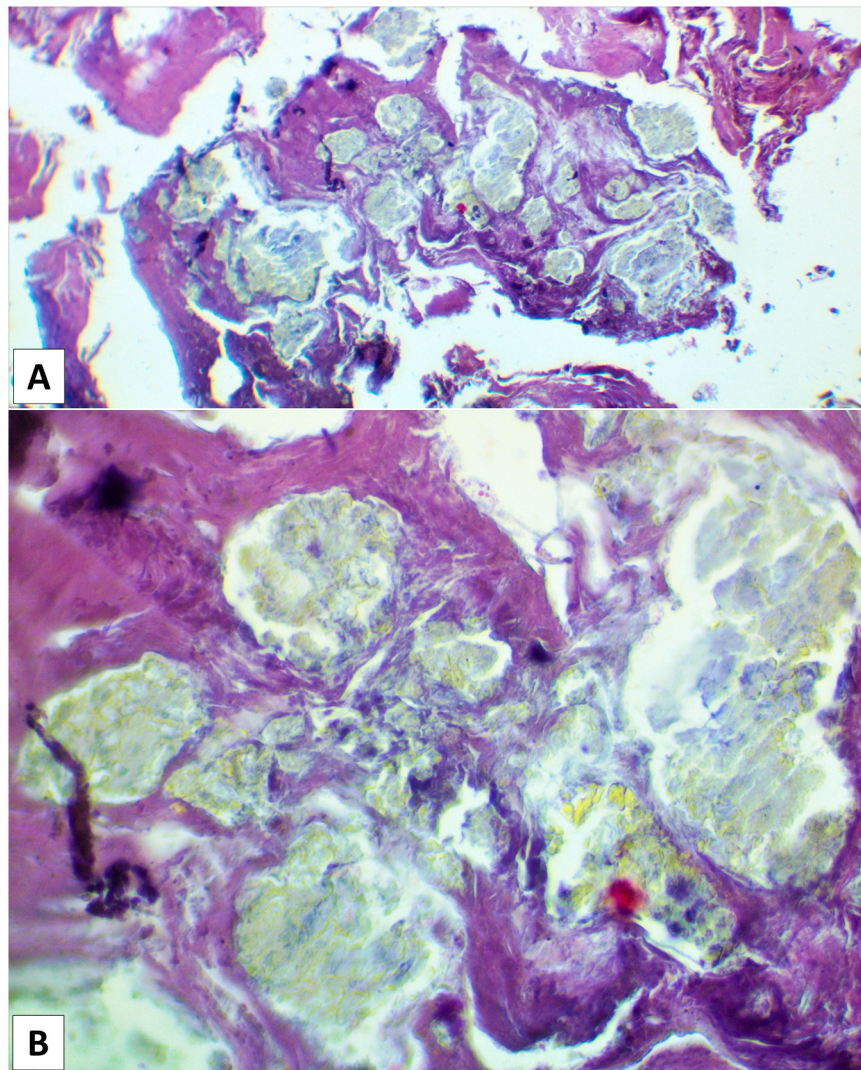


Fig. 4. A. Fibrocalcified pulmonary nodes with some small lacunae surrounded by fibrous tissue containing necrotic, caseous, material (HE 2,5X). B. Detail at higher magnification (HE, 20X).

pneumoconiosis, calcified nodules are less common but can occur (Khan et al., 2010). Since the features of calcifications from the lungs of individual Comiso 21 are different, these diseases were ruled out.

Finally, the hypothesis of a calcification of a previous Ghon complex of an apical nodular tuberculosis called Ranke complex can be considered (Corrin and Nicholson, 2011). Calcified nodules due to tuberculosis often appear as discrete, well-defined, and sharply marginated nodules. The calcification tends to be dense and uniform, frequently located in the upper lobes or apices of the lungs.

It can be hypothesized that the Sicilian friar came into contact with *Mycobacterium* and later developed the primary infection (Delogu et al., 2013). Tuberculosis in past populations is a well-investigated topic, both epidemiologically and paleopathologically (see Bañuls et al., 2015; Spekker et al., 2024; Gémes et al., 2023; Zink et al., 2023; Dutour, 2023). Mummies are a precious find, as they can exhibit the signs of a disease not only in the bones, but also in the residual internal organs, soft tissues, and calcifications of parenchymatous structures. For example, in a 350-year-old female mummy of the Joseon Culture (South Korea), Kim et al. (2016) described six calcified nodules in right-sided lung detected with CT scans. Based only on radiological patterns, the authors excluded primary lung cancer, carcinoid, pulmonary metastases and hamartoma. The differential diagnosis narrows to infectious diseases like TB, and the authors argue that the observed patterns align

with clinical findings from modern patients. Moreover, other diagnoses of tuberculosis obtained by histological study are described in the literature. At the site of Dolmen des Peirieres (Villedubert, Aude, France) from the Chalcolithic period, histological analyses suggested that a solid nodule from a collective burial may be interpreted as a lymph node with calcified areas due to tuberculosis (Baud and Kramer, 1991). More recently, Nerlich and colleagues studied the mummy of Count Heinrich LII. Reuss-Köstritz (1763–1851 CE); the histological slides of the samples obtained from the lungs showed a healed pulmonary tuberculosis confirmed by ancient DNA analyses (Nerlich et al., 2021).

Other investigators have also successfully extracted ancient DNA from mummified tissues, confirming the high prevalence of tuberculosis in 18th–19th-century populations. For example, Fletcher et al., 2003 examined samples from 168 Hungarians mummies from 1731 to 1838 with PCR amplification, discovering that 55 % of the individuals were infected with *M. tuberculosis* complex. Molecular studies have also provided comparisons of the phylogeny of *M. tuberculosis* complex from the 18th century, finding in some cases (Kay et al., 2015) that it is the same strain (termed Lineage 4), which still infects millions of patients today, mainly in Europe and America.

The mummies of Comiso represent an interesting sample of a portion of the Sicilian population between the 18th and 19th centuries. It is not surprising to find a case of tuberculosis, since the subject lived at a time

during which the disease was widespread; indeed, the high incidence of TB during the Modern Age is well-known (Murray, 2004; Daniel, 2006). In Sicily, there are other known cases of tuberculosis identified in ancient human remains, such as two male mummies from Modica (18th-19th century) (Ventura et al., 2016) and one female mummy from Scicli (second half of the 19th century) (Ventura et al., 2022), diagnosed by CT scanning in both cases. These cases demonstrate that tuberculosis was a common infectious disease on the island prior to the antibiotic era. The peak of tuberculosis was recorded at the beginning of the 19th century, as it represented the most common cause of death, reaching a rate of about 28.7 cases/10,000 inhabitants in the largest cities of the Kingdom of Italy from 1887 to 1901 (Della Peruta, 1980).

Tuberculosis has been a major health concern throughout human history, and its presence in historical populations provides insights into the epidemiology and evolutionary history of the disease (Barbier and Wirth, 2016). By studying ancient cases like the one presented in this paper, it is possible to trace the evolution of tuberculosis, understand its transmission dynamics in different historical contexts, and potentially correlate disease patterns with social and environmental factors of the time. These insights are crucial for understanding the long-term impact of tuberculosis on human populations. The methods employed in this study, including radiological imaging, histopathological analysis, and molecular techniques, explores the diverse and multifaceted fields in which palaeopathology operates. These interdisciplinary approaches not only allow for the diagnosis of ancient diseases but also facilitate the reconstruction of paleoepidemiological profiles, thus bridging the gap between medical sciences and archaeology.

As for the identification of the diffuse and intense anthracosis in the friar of Comiso, this is one of the most frequent microscopic findings in mummy paleopathology (Isidro et al., 2014; Paudice et al., 2021; Grove et al., 2015; Vellone et al., 2015). The inhalation of soot and smoke of the chimneys to cook food and to heat the rooms, constant for all social classes, provoked the onset of anthracosis (Paudice et al., 2021).

The collection of mummies of Comiso is of extraordinary importance since it may reveal the health status of a small population of the recent past. The investigations on these bodies are still in progress and paleopathological data could be integrated with historical and archival studies in order to understand the lifestyle and the health status of this population.

CRedit authorship contribution statement

Raffaele Gaeta: Writing – original draft, Methodology, Investigation. **Frank Maixner:** Writing – original draft, Methodology, Investigation, Formal analysis. **Valentina Giuffra:** Writing – review & editing, Validation, Supervision, Conceptualization. **Antonio Fornaciari:** Writing – review & editing, Visualization, Investigation. **Giacomo Aringhieri:** Methodology, Investigation.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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