Acta Biologica Szegediensis

Volume 64(2):99-110, 2020
Acta Biologica Szegediensis
http://abs.bibl.u-szeged.hu/index.php/abs

DOI:10.14232/abs.2020.2.99-110

ARTICLE

Lipid biomarker-based verification of TB infection in mother's and daughter's mummified human remains (Vác Mummy Collection, 18th century, CE, Hungary)

Orsolya A. Váradi[1,2]*, Ildikó Szikossy[1,3], Olga Spekker[1], Dávid Rakk[2], Gabriella Terhes[4], Edit Urbán[5,6], William Berthon[1], Ildikó Pap[1,3,7], Frank Maixner[8], Albert Zink[8], Csaba Vágvölgyi[2], Helen D. Donoghue[9], David E. Minnikin[10], György Pálfi[1#], András Szekeres[2#]

[1]Department of Biological Anthropology, Faculty of Science and Informatics, University of Szeged, Hungary.
[2]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary.
[3]Department of Anthropology, Hungarian Natural History Museum, Budapest, Hungary.
[4]Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged, Szeged, Hungary.
[5]Department of Medical Microbiology and Immunology, Medical School, University of Pécs, Pécs, Hungary.
[6]Institute of Translational Medicine, Medical School, University of Pécs, Pécs, Hungary.
[7]Department of Biological Anthropology, Eötvös Loránd University, Budapest, Hungary.
[8]Institute for Mummy Studies, Eurac Research, Bolzano, Italy.
[9]Centre for Clinical Microbiology, University College London, London, UK.
[10]Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, UK.

ABSTRACT The perpetual burden of tuberculosis (TB) keeps drawing the focus of research on this disease. Among other risk factors (e.g., poor living conditions, malnutrition, smoking, HIV infection, etc.), being in close contact with a TB infected person requires special attention. For a better understanding of the disease, paleopathological investigations concerning TB have been carried out with various techniques for a long a time; nevertheless, analysis of incidence among family members is hardly possible in past populations. An exceptional group of naturally mummified individuals, the collection of the Vác mummies (Hungary, 18th century CE), is known about the large TB incidence rate, which has been revealed by aDNA analysis. Besides the high rate of TB infection, another interesting aspect of the collection is that in some cases, the family connections could be reconstructed. In this paper, we present the mycocerosic acid profiles gained by HPLC-HESI-MS measurements of two Vác mummies, who were mother and daughter according to the personal records. Earlier metagenomic analysis already revealed mixed *M. tuberculosis* infection with the same bacterial strains in both individuals; moreover, the same bacterial strains were recorded in both cases.

Acta Biol Szeged 64(2):99-110 (2020)

KEY WORDS
high-performance liquid chromatography
lipid biomarkers
mass spectrometry
mummies
mycocerosates
tuberculosis

ARTICLE INFORMATION
Submitted
15 December 2020.
Accepted
29 December 2020.
***Corresponding author**
E-mail: varadi.orsolya.90@gmail.com

Introduction

Tuberculosis (TB) is a well-known infectious disease, which can be caused by members of *Mycobacterium tuberculosis* complex (MTBC), in humans, and also several animal species (Brites et al. 2018; WHO 2020). The 12 species/varieties, included in the complex, are genetically closely related, presenting 99.9% similarity in the nucleotide level (Brosch et al. 2002; Brites and Gagneux 2017; Brites et al. 2018; Riojas et al. 2018). Up to now, the following species are considered as members of the complex: *M. tuberculosis, M. africanum, "M. canettii", M. bovis, M. microti, M. pinnipedii, M. orygis, M. mungi, M. suricattae, M. caprae,* "chimpanzee bacillus", and "dassie bacillus" (Brites et al. 2018; Riojas et al. 2018), but only the first three are specifically human pathogens (Castets et al. 1968; Brosch et al. 2002; Niemann et al. 2004; Bañuls et al. 2015). In human infections, the most common scenario is when transmission occurs via inhalation of bacilli-filled droplets, which are projected in the air while coughing, sneezing or even talking (Flynn and Chan 2001; Bañuls et al. 2015; Getahun et al. 2015).

Several risk factors contribute to the spread of TB: some of them affect the transmission itself (e.g. poor living and working condition, household overcrowding, etc.), and some increase the host's susceptibility (e.g., HIV infection, malnutrition, smoking, diabetes) (Lönnroth et al. 2009; WHO 2020). To reduce TB burden, the prevention of new infections is crucial, and a key factor is

#The two last authors contributed equally.

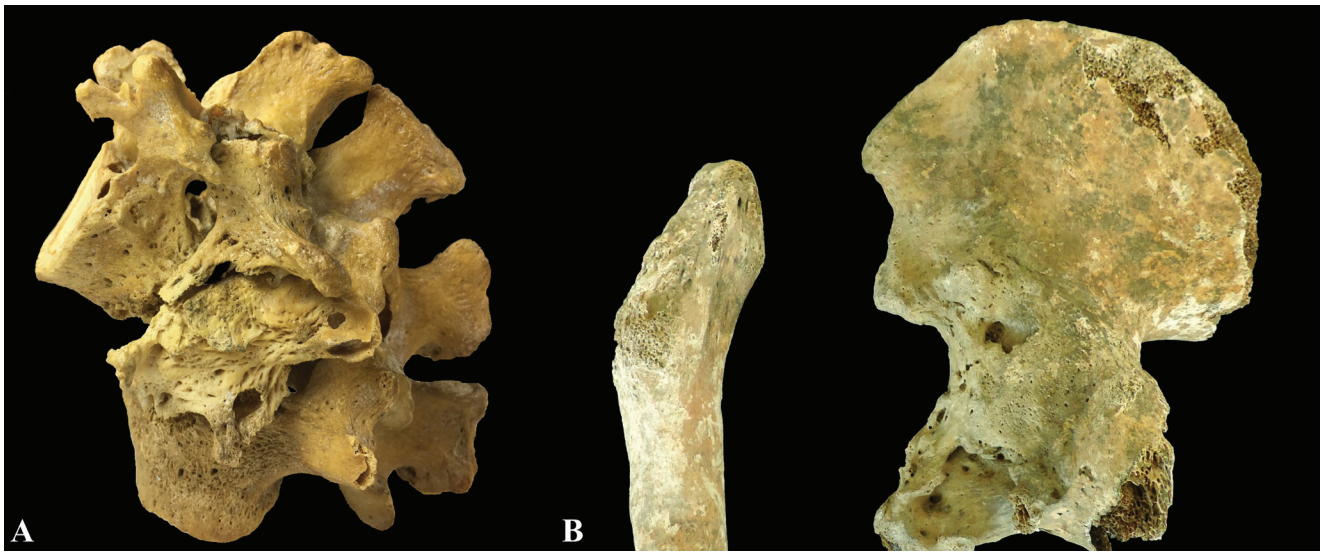

Figure 1. Examples of osteoarticular TB associated lesions. **A:** Pott's gibbus (Robert J. Terry Anatomical Skeletal Collection, Terry No. 1124R). **B:** *Coxitis tuberculosa* (Bélmegyer–Csömöki domb, Grave No. 90).

providing TB preventive treatment for high-risk groups (WHO 2020). Being in close contact with a person who has active TB – especially in newly diagnosed TB patients – means a high risk for transmission; thus, close family members, principally those from the same household, need particular attention (Vidal et al. 1997; Wang and Lin 2000; Horsburgh and Rubin 2011; Augustynowicz-Kopeć et al. 2012; Acuña-Villaorduña et al. 2018; WHO 2020).

According to WHO estimates, approximately 1.2 million HIV-negative and an additional 208000 HIV-positive people died of TB and about 10 million people fell ill with the disease globally in 2019 (WHO 2020). The relatively high incidence, the appearing rifampicin- and multidrug-resistant TB (MDR-TB) strains and the co-infection cases (especially with HIV) highlight the necessity of extensive TB research. This disease is mainly remembered due to its devastating effects during the 18th - 19th century, when it was highly spread in Europe, implying an extraordinary burden (Bello et al. 1999; Vuorinen 1999, Glaziou et al. 2018; Loddenkemper et al. 2018; Roberts 2020), but has been a recognised threat for thousands of years (Gutierrez et al. 2005; Daniel 2006; Baker et al. 2015; Barberies et

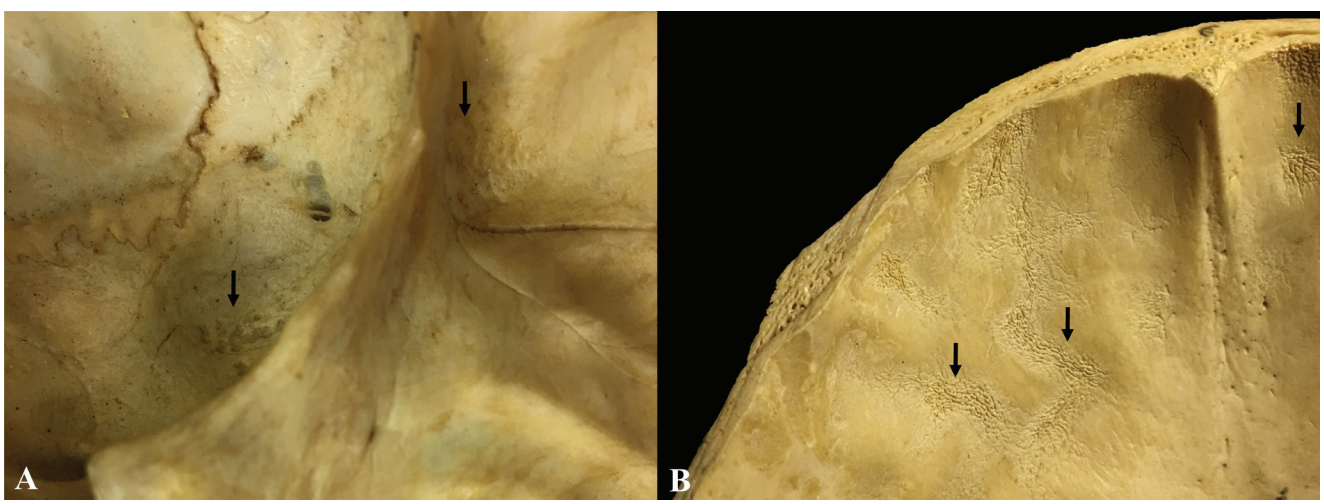

Figure 2. Examples of TBM associated lesions. **A:** Granular impressions on the greater wing of the sphenoid bone (Robert J. Terry Anatomical Skeletal Collection, Terry No. 566). **B:** Abnormal blood vessel impressions on the endocranial surface of the frontal bone (Robert J. Terry Anatomical Skeletal Collection, Terry No. 254).

al. 2017). To achieve a better understanding of the evolution of the infectious agent, paleopathological research investigating the epidemiology of TB in past populations is highly important.

The paleopathological signs of TB include Pott's gibbus (Fig. 1A) and *coxitis tuberculosa* (Fig. 1B), traces of cold abscess, and endocranial lesions caused by tuberculous meningitis (TBM) e.g. granular impressions (Fig. 2A), and abnormal blood vessel impressions (Fig. 2B) (Schultz 1993, 1999, 2001, 2003; Aufderheide and Rodríguez-Martín 1998; Marcsik et al., 1999; Pálfi and Marcsik 1999; Hershkovitz et al. 2002; Maczel 2003; Ortner 2003; Pálfi and Molnár 2009; Pálfi et al. 2012, 2015; Spekker et al. 2012; Kajdocsi Lovász 2015; Masson et al. 2015; Molnár et al. 2015; Paja et al. 2015; Schultz and Schmidt-Schultz 2015; Spekker 2018; Spekker et al. 2020a, 2020b). Moreover, new bone formation on the long bones and on the visceral surface of ribs are used as TB-related markers (Roberts et al. 1994; Marcsik et al. 2009; Santos and Roberts 2001, 2006; Hershkovitz et al. 2002; Maczel 2003; Matos and Santos 2006; Pálfi and Molnár 2009; Pálfi et al. 2012, 2015; Kajdocsi Lovász 2015; Masson et al. 2015; Molnár et al. 2015). Since skeletal TB and CNS TB develop in only a few cases (Golden and Vikram 2005; Rock et al. 2008; Spekker et al. 2018; Rodriguez-Takeuchi et al. 2019; Spekker et al. 2020a; Spekker et al. 2020b), the simultaneous application of molecular biological and analytical techniques are useful tools to draw a clearer picture about the paleoepidemiology of TB (Molnár et al. 2015; Pálfi et al. 2015; Donoghue et al. 2017). Since the 1990s, two approaches are commonly applied to supplement the morphological TB-related paleopathological investigations, namely aDNA based and lipid biomarker-based methods (Spigelman and Lemma 1993; Donoghue et al. 1998; Gernaey et al. 1998; Hershkovitz et al. 2008; Redman et al. 2009; Chan et al. 2013; Kay et al. 2015; Donoghue et al. 2017).

The lipid biomarker-based methods benefit from the lipid-rich cell wall, characteristic of mycobacteria (Minnikin and Goodfellow 1980; Minnikin 1982; Daffé and Lanéelle 1988; Minnikin et al. 1993; Hershkovitz et al. 2008; Redman et al. 2009; Lee et al. 2012; Minnikin et al. 2015a; Donoghue et al. 2017). Most commonly, the mycolic acid (MA) and mycocerosic acid (MC) components, and the C27 mycolipenic acid are used. MAs, MCs, and mycolipenic acids can be found in the so-called Mycobacterial Outer Membrane (MOM) (Minnikin et al. 2015b). MAs are long chain α-alkyl-β-hydroxy fatty acids, which are covalently bound to the mycoloylarabinogalactan-peptidoglycan macromolecules (Watanabe et al. 2001; Minnikin et al. 2015b; Abrahams and Besra 2016; Batt et al. 2020; Dulberger et al. 2020). MCs are long-chain multimethyl-branched-chain fatty acids esterified mainly with phthiocerol and phenolphthiocerol long-chain diols (Minnikin 1982; Daffé and Lanéelle 1988; Redman et al. 2009; Minnikin et al. 2015b; Batt et al. 2020). In contrast with MAs, MCs can be found only in a smaller group of mycobacteria, namely in *M. tuberculosis*, *M. bovis*, *M. gastri*, *M. haemophilum*, *M. kansasii*, *M. leprae*, *M. marinum,* and *M. ulcerans* (Draper et al. 1983; Minnikin et al. 1985; Daffé and Lanéelle 1988; Hartmann and Minnikin, 1992; Minnikin et al. 1993; Redman et al. 2009). MCs have been detected for paleopathological investigations traditionally via NICI-GCMS (Redman et al. 2009) and a HPLC-MS method has been newly introduced (Váradi et al. 2021).

Figure 3. Dominican Church of Vác. Photo taken by András Thumbasz.

In 1994, a group of naturally mummified individuals were found in a long-forgotten crypt during the renovation of the Dominical Church of Vác (1994–1995) (Fig. 3) (Pap et al. 1999). The discovered Vác mummy collection is well-documented, with many available individual data (Szikossy et al. 1997). The mummies are curated in the Department of Anthropology, Hungarian Natural History Museum, Budapest, Hungary. The collection is known for the high presence of TB infected cases, that drew the focus of several studies on this group (Szikossy et al. 1997; Pap et al. 1999; Fletcher et al. 2003; Donoghue et al. 2011; Chan et al. 2013; Kay et al. 2015; Pap et al. 2017). Most of these individuals lived in the 18th century CE, which is from the pre-antibiotic era; therefore, they represent a well-characterised link between recent and archaeological samples. Besides the broad pathological investigations, demographical analysis and exploration of family connections were carried out, as well as attempts to reconstruct individual life stories (Kustár et al. 2011a, 2011b; Szikossy et al. 2015; Szikossy 2020).

The aim of this study is to present and compare the results of mycocerosic acid profiling of two TB infected Vác mummies, a mother (Fig. 4A) and her daughter (Fig.

4B). Earlier both individuals were proved to have mixed infection with the same *M. tuberculosis* strains via aDNA analysis (Kay et al. 2015), and samples taken from the daughter presented positive MC profiles in previous HPLC-MS measurements (Váradi et al. 2021).

Materials and Methods

MTBC strains and mummy samples used in this study

For reference, in an earlier study, we used five MTBC strains (laboratory IDs of the isolated strains MTBC-1/2015; MTBC-254/2000; MTBC-3910/2014; MTBC-242/2000; and MTBC-1/8508/2014), isolated from patients, who had been diagnosed with pulmonary tuberculosis. The average distribution of the reference strains has been evaluated and published in our earlier report (Váradi et al. 2021). The isolation of the reference strains was carried out in the Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary and the National Korányi Institute of TB and Pulmonology, Budapest, Hungary, according to the national recommendations (EMMI, State Secretariat for Healthcare, 2018). The identification and growing conditions followed a previously described protocol (Váradi et al. 2021). The harvested bacterial samples were stored in freeze-dried form at -20 °C.

The examined human sample was taken from the chest region of the late Anna Schőner (body number: #28, inventory number: 2009.19.28., age at death: 55 years). The rib sample was removed by sanitized tweezers and stored in a tightly closed bag at room temperature. The bone was powdered in the clean laboratory of the Institute for Mummy Studies, EURAC Research, Bolzano, Italy.

Sample preparation and instrumental analysis

In the case of the sample pre-treatment and measurement of the MTBC strains, 20 mg of bacterial material was utilized and the previously described method was applied (Váradi et al. 2021). For the lipid analysis of the mummy sample, 434 mg of bone powder was used. The sample pre-treatment was carried out briefly as follows: the samples were heated at 100 °C overnight with the addition of 20% KOH in MeOH (2 mL; m/V) and toluene (1 mL) in PTFE capped glass tubes. Samples were acidified to pH 1 with the addition of 10% HCl and 37% HCl solutions. Thereafter, the samples were extracted with the addition of toluene (1 mL) three times, and one more time with the addition of hexane isomer mixture (1 mL). The removed and combined organic layers were evaporated to dryness in vacuum with a Savant SC250EXP SpeedVac concentrator (Thermo Scientific, Waltham, Massachusetts, USA). The mummy sample was dissolved in 1000 µl and the bacterial samples were dissolved in 200 µL of the following mixture: isopropanol (IPA):heptane:acetonitrile (MeCN) (4:1:5). The sample solutions were filtered by PTFE syringe filters (pore size: 2.0 µm; diameter: 13 mm).

The measurements were carried out on a Dionex Ultimate 3000 UHPLC system (Thermo Scientific, Waltham, Massachusetts, USA), which was coupled with a Q-Exactive Plus (Thermo Scientific, Waltham, Massachusetts, USA) mass spectrometer (MS). For the separation, a Gemini – NX C18 (3 µm, 110A, 50 mm x 2 mm) column (Phenomenex, Torrance, California, USA) was used at 30 °C.

The separation was carried out with gradient elution

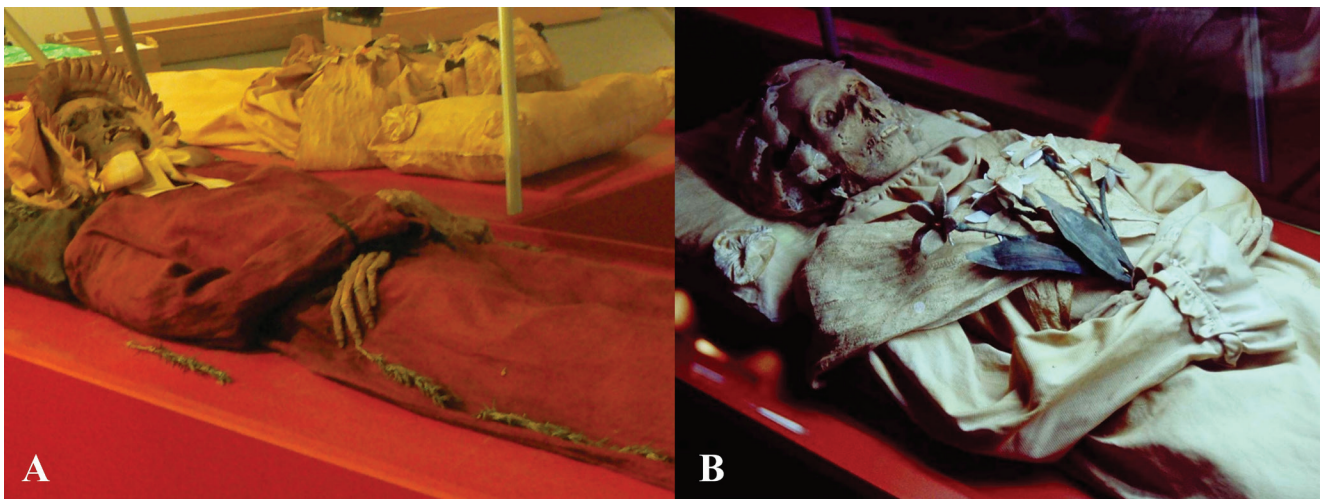

Figure 4. Sources of the mummy samples. **A:** Anna Schőner (Body No: 28, Inv. No: 2009.19.28.). **B:** The late Terézia Hausmann (Body No: 68, Inv. No: 2009.19.68.). Photos were taken on the exhibition of the Hungarian Natural History Museum.

Table 1. Gradient elution program applied for the HPLC separation of mycocerosic acids.

Time (min)	Eluent A (%): MeCN + 0.1% acetic acid	Eluent B (%): IPA:heptane (8:2) + 0.1% acetic acid
0	90	10
5	85	15
15	30	70
16	10	90
18	10	90
18.5	80	20
19.5	80	20
20	10	90
23	10	90
24	90	10
28	90	10

(see details in Table 1), and 200 µL/min flow rate was applied. The injection needle was thoroughly washed before and after injection, with 150 µL IPA:heptane (8:2). Solvent blank injections were made between each sample investigation.

Regarding the MS parameters, the following settings were applied: the sheath gas flow and the auxiliary gas rates were set to 35 L/min and 10 L/min, respectively, the capillary and the auxiliary gas was heated to 350 °C, the spray voltage was set to 4 kV, while the S-lens voltage was 70 V. The targeted MCs (Fig. 5) were monitored in negative Selected Ion Monitoring (SIM) mode: 409.40510 (C27, $C_{27}H_{53}O_2$), 437.43640 (C29, $C_{29}H_{57}O_2$), 451.45205 (C30, $C_{30}H_{59}O_2$), 479.48335 (C32, $C_{32}H_{63}O_2$), and 493.49900 (C33, $C_{33}H_{65}O_2$), with 0.4 *m/z* isolation window. The area values of the MC peaks were calculated with TraceFinder 4.0 General Quan Software (Thermo Scientific, Waltham, Massachusetts, USA). The maximum mass deviation from the calculated $[M\text{-}H]^-$ ion mass was 5 ppm for peak identification. The detected MC peaks were normalised to the peak with the highest area value.

Results and Discussion

Three members of the Hausmann family were identified in the Vác Mummy Collection of the Hungarian Natural History Museum. The mother, Anna Schőner (1738-1793) and her two daughters, Terézia (1769-1797) and Barbara Hausmann (1780-1795). Their father, János Hausmann is not in the collection. According to the aDNA examinations (Fletcher et al. 2003; Chan et al. 2013; Kay et al. 2015), all three female members of the Hausmann family were infected by tuberculosis. First, Anna Schőner died at the age of 55 in 1793, December 16. Two years later, she was followed by her younger daughter, Barbara Hausmann, who died at the age of 15, on March 2, 1975. In another two years, the elder daughter, Terézia Hausmann passed away at the age of 28, on December 25, 1797 (Exploration documentation of the Dominican Church of Vác, 1994-1995, Tragor Ignác Múzeum.). Both girls were emaciated, suggesting a long-lasting illness. It is possible that the thirteen-year-older Terézia took care of her sister before the illness made her too weak (Cseplák et al. 2015, Donoghue et al. 2021). In this study, we present the mycocerosate profiles observed by the analysis of samples taken from Anna Schőner (#28) and Terézia Hausmann (#68).

The lipid profile observed for the extract of the rib sample taken from the #28 individual is shown in Fig. 6A and Fig. 7. The *M. tuberculosis* C27 MC minor component was not detected in the extract. In the MC profile, the main peak was the C32 (100), and it was accompanied by a relatively high peak of C30 (49). The C29 MC was presented in a relatively high ratio (25), as well. The C33 MC was also detectable but was a minor component in the extract (3). The first eluting component was the C29 MC with the retention time of 8.20 min, followed by the C30 and C32 MCs at 8.75 and 10.64 min, respectively. The retention time of the C33 MC peak was 10.97. The general distribution of the detected MCs was in accord with others from the same group of mummies, and with the average profile gained by the analysis of clinical samples (e.g., Fig. 5B, Fig. 6B), and with the MTB profiles published using different approaches (Minnikin et al. 1993; Redman et al. 2009; Váradi et al. 2021). Based on our previous results, the observed lipid profile fulfills the requirement to be identified as a positive case, as the three most presented MCs were the C32, C30, and C29, with a principal amount of the C32 component (Fig. 6C, Fig. 7). The samples belonging to the daughter (#68) of this individual were found earlier to be positive with HPLC-MS analysis (Váradi et al. 2021). In that case, both soft tissue and rib samples were included in the investigation. The MC profile of the soft tissue presented provided clean peaks for C32, C29, C30 and C33 MCs. The main peak was the C32 (100), which was accompanied by major C29 (38) and C30 (97) peaks and with C33 MC (5) as minor

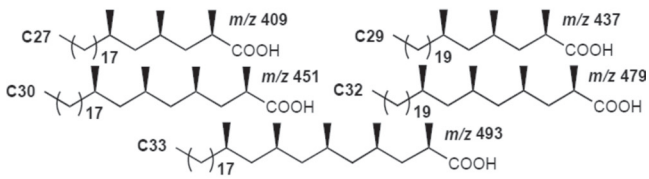

Figure 5. Structures of mycocerosic acids. The mass spectral *m/z* values correspond to carboxylate anions ($M - H^+$).

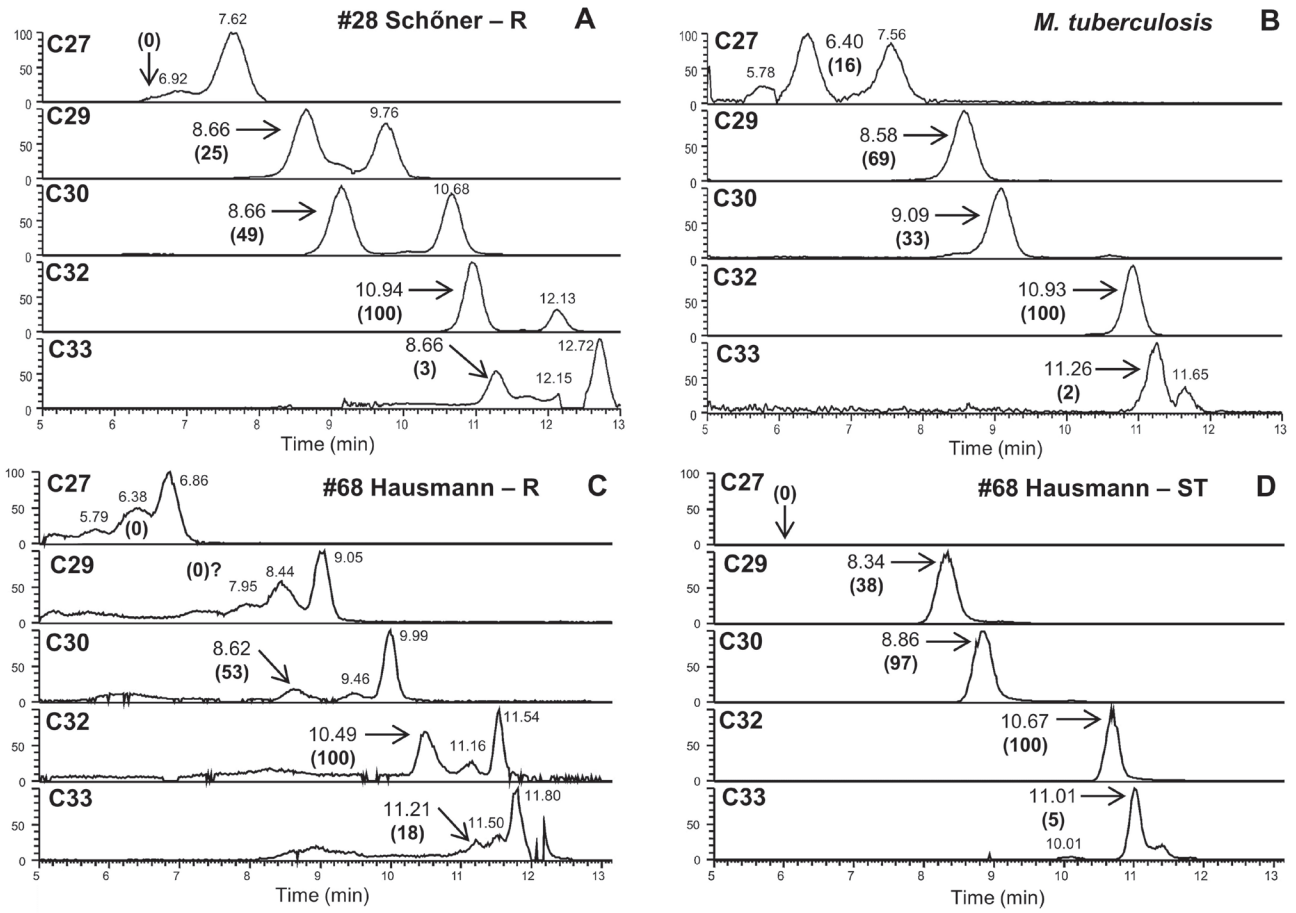

Figure 6. Mycocerosic acid profile of M. tuberculosis and positive mycocerosate (MC) profiles from mummy samples #28 and #68. **A:** Rib (R) sample from Anna Schőner (#28). **B:** Mycocerosate profile of M. tuberculosis 254-2000. **C** and **D:** Rib (R) and Soft Tissue (ST) samples from Terézia Hausmann (#68). MC distributions are shown normalised to the main component (100).

component (Fig. 6D, Fig. 7). The extract of the rib sample of #68 individual had clear main C32 (100) and major C30 components (53), but the minor C33 was indistinct (~18) and the area for the expected C29 was obscured.

Samples taken from the same individuals were earlier analysed with two different aDNA techniques. Firstly, as part of an extensive study, the examination of over 350 samples covering 168 individuals were screened for the 123-bp region of the IS*6110* insertion sequence (Fletcher et al. 2003). The positive samples were investigated for silent point mutations of the *gyrA* 95 and *katG* 463 genes; for the differentiation of the infectious agents into three genotypes, following the work of Sreevatsan et al. (1997). Our positive MC profile matched the result of the DNA-based analysis; traits of *gyrA* 95 and *katG* 463 genes were found in the abdomen, with the silent point mutations characteristic to the group 2 genotype of *M. tuberculosis* (Fletcher et al. 2003). In the case of the same study, during the analysis of the samples taken from the #68 individual's left chest, the silent point mutations of the *gyrA* 95 and *katG* 463 genes were characteristic of the group 3 genotype of *M. tuberculosis*. Metagenomic analysis carried out on the samples taken from the abdomen region of the #28 individual and on the samples taken from the left chest region of the #68 individual presented similar results (Kay et al. 2015). In both cases, a mixed MTB infection was revealed, but although the detected genotypes were the same, they were detected in different proportions. One of the *M. tuberculosis* strains belonged to sublineage 4.1.2.1, also known as the Haarlem lineage (Kay et al. 2015; Stucki et al. 2016), and the other was assigned by Kay et al. (2015) to sublineage 4.7, which is currently part of sublineage 4.10 according to Stucki et al. (2016). The metagenomic results suggest a transmission between mother and child or infection from the same source (Kay et al. 2015).

Although in this case the applied MC profiling method cannot provide the same resolution regarding the *M.*

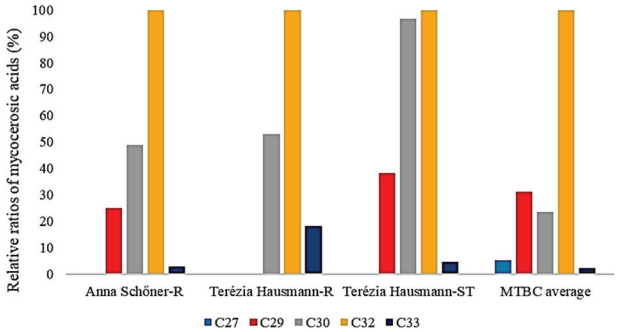

Figure 7. The normalised distribution of positive mycocerosate profiles from mummy rib (R) and soft tissue (ST) samples. Also shown is an "MTB average", calculated in previous examinations of clinical samples.

tuberculosis strains, it demonstrates the widespread occurrence of TB infection among the Vác mummies. The combined application of morphology, aDNA and lipid biomarker analysis is important to gain a clearer picture in paleopathological practice as the results can support each other, and help to reveal positive cases, which would possibly remain hidden if only one method was applied (Hershkovitz et al. 2008; Lee et al. 2012; Baker et al. 2015; Masson et al. 2015; Molnár et al. 2015; Donoghue et al. 2017; Luna et al. 2020). The examination of mummified human remains with a variety of different approaches is especially useful and provides a unique insight to TB research (Salo et al. 1994; Zink et al. 2003; Donoghue et al. 2004; Donoghue et al. 2009; Minnikin et al. 2011; Chan et al. 2013; Lalremruata et al. 2013; Kay et al. 2015; Piombino-Mascali et al. 2015; Szikossy et al. 2015).

Acknowledgements

David E. Minnikin acknowledges Leverhulme Trust Project Grant F/00 094/BL and UK Medical Research Council Programme Grant MR/S000542/1. We acknowledge the support of the National Research, Development and Innovation Office (Hungary) (grant number: K 125561). We also acknowledge the support for establishing the infrastructural background (grant number: GINOP-2.3.3-15-2016-00006).

References

Abrahams KE, Besra GS (2016) Mycobacterial cell wall biosynthesis: a multifaceted antibiotic target. Parasitology 145(2):116-133.

Acuña-Villaorduña C, Jones-López EC, Fregona G, Marques-Rodrigues P, Gaeddert M, Geadas C, Hadad DJ, White LF, Molina LPD, Vinhas S, Ribeiro-Rodrigues R, Salgame P, Palaci M, Alland D, Ellner JJ, Dietze R (2018) Intensity of exposure to pulmonary tuberculosis determines risk of tuberculosis infection and disease. Eur Respir J 51:1701578.

Aufderheide AC, Rodríguez-Martín C (1998) The Cambridge encyclopedia of human paleopathology. Cambridge University Press, Cambridge, UK; 118-141.

Augustynowicz-Kopeć E, Jagielski T, Kozińska M, Kremer K, van Sooligen D, Bielecki J, Zwolska Z (2012) Transmission of tuberculosis within family-households. J Infect 64: 596-608.

Baker O, Lee OY-C, Wu HHT, Besra GS, Minnikin DE, Llewellyn G, Williams CM, Maixner F, O'Sullivan N, Zink A, Chamel B, Khawam R, Coqueugniout E, Helmer D, Le Mort F, Perrin P, Gourichon L, Dutailly, Pálfi Gy, Coqueugniot H, Dutour O (2015) Human tuberculosis predates domestication in ancient Syria. Tuberculosis 95(Suppl.1):S4-S19.

Bañuls A-L, Sanou A, Nguyen TVA, Godreuil S (2015) Mycobacterium tuberculosis: ecology and evolution of human bacterium. J Med Microbiol. 64(11):1261-1269.

Barberis I, Bragazzi NL, Galluzzo L, Martini M (2017) The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus. J Prev Med Hyg 58:E9-E12.

Batt SM, Minnikin DE, Besra GS (2020) The thick waxy coat of mycobacteria, a protective layer against antibiotics and the host's immune system. Biochem J 477(10):1983-2006.

Bello S, Signoli M, Maczel M, Dutour O (1999) Evolution of mortality due to tuberculosis in France (18-20th centuries). In Pálfi Gy, Dutour O, Deák J, Hutás I, Eds., Tuberculosis Past and Present. TB Foundation: Szeged, Hungary & Golden Book Publisher, Budapest, Hungary, 95-104.

Brites D, Gagneux S (2017) The nature and evolution of genomic diversity in the *Mycobacterium tuberculosis* complex. In Gagneux S, Ed., Strain Variation in the *Mycobacterium tuberculosis* Complex: Its Role in Biology, Epidemiology and Control. Advances in Experimental Medicine and Biology 1019. Springer International Publishing, Cham, Switzerland, 1-26.

Brites D, Loiseau C, Menardo F, Borrell S, Boniotti MB, Warren R, Dippenaar A, Parsons SDC, Beisel C, Behr MA, Fyfe JA, Coscolla M, Gagneux S (2018) A new phylogenetic framework for the animal-adapted *Mycobacterium tuberculosis* complex. Front Microbiol 9:2820.

Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, Garnier T, Gutierrez C, Hewinson G, Kremer K, Parsons LM, Pym AS, Samper S, van Soolingen D, Cole ST (2002) A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. PNAS 99(6):3684-3689.

Castets M, Boisvert H, Grumbach F, Brunel M, Rist N (1968)

Tuberculosis bacilli of the African type: preliminary note. Rev Tuberc Pneumol 32:179-184.

Chan JZ-M, Sergeant MJ, Lee OY-C, Minnikin DE, Besra GS, Pap I, Spigelman M, Donoghue HD, Pallen MJ (2013) Metagenomic analysis of tuberculosis in a mummy. N Engl J Med 369:289-290.

Cseplák Gy, Szikossy I, Pap I (2015) A váci múmiákról. Antropo-medicinális tanulmányok 52 váci múmia vizsgálatáról egy bőrgyógyász fényképes jegyzeteivel. Semmelweis Kiadó és Multimédia Stúdió Kft., Budapest.

Daffé M, Lanéelle MA (1988) Distribution of phthiocerol diester, phenolic mycosides and related compounds in mycobacteria. J Gen Microbiol 134(7):2049-2055.

Daniel TM (2006) The history of tuberculosis. Respir Med 100:1862-1870.

Donoghue HD, Lee OY-C, Minnikin DE, Besra GS, Taylor JH, Spigelman M (2009) Tuberculosis in Dr Granville's mummy: a molecular re-examination of the earliest known Egyptian mummy to be scientifically examined and given a medical diagnosis. Proc R Soc B 27751-2776.

Donoghue HD, Pap I, Szikossy I, Spigelman M (2011) Detection and characterization of *Mycobacterium tuberculosis* DNA in 18th century Hungarians with pulmonary and extra-pulmonary tuberculosis. In Gill-Frerking H, Rosendahl W, Zink A, Piombino-Mascali D, Eds., Yearbook of Mummy Studies, Verlag Dr. Friedrich Pfeil, München, Germany, 1:51-56.

Donoghue HD, Spigelman M, Greenblatt CL, Lev-Maor G, Bar-Gal GK, Matheson C, Vernon K, Nerlich AG, Zink AR (2004) Tuberculosis: from prehistory to Robert Koch, as revealed by ancient DNA. Lancet Infect Dis 4(9):584-592.

Donoghue HD, Spigelman M, Zias J, Gernaey-Child AM, Minnikin DE (1998) *Mycobacterium tuberculosis* complex DNA in calcified pleura from remains 1400 years old. Lett Appl Microbiol 27:265-269.

Donoghue HD, Taylor MG, Stewart GR, Lee OY-C, Wu HHT, Besra GS, Minnikin DE (2017) Positive diagnosis of ancient leprosy and tuberculosis using ancient DNA and lipid biomarkers. Diversity 9(4):46.

Donoghue HD, Pap I, Szikossy I, Spigelman M (2021) The Vác Mummy Project: Investigation of 265 eighteenth-century mummified remains from the TB pandemic era. In Shin DH, Bianucci R, Eds., The Handbook of Mummy Studies. Springer, Singapore.

Draper P, Payne SN, Dobson G, Minnikin DE (1983) Isolation of a characteristic phthiocerol dimycocerosate from *Mycobacterium leprae*. J Gen Microbiol 129(3):859-863.

Dulberger CL, Rubin EJ, Boutte CC (2020) The mycobacterial cell envelope – a moving target. Nat Rev Microbiol 18:47-59.

EMMI, State Secretariat for Healthcare (2018) Egészségügyi szakmai irányelv – A tuberkulózis prevenciójáról, diagnosztikájáról, terápiájáról és gondozásáról. Available: https://www.hbcs.hu/uploads/jogszabaly/2838/fajlok/EMMI_szakmai_iranyelve_tuberkulozis.pdf; 2014. Accessed 01 August 2020.

Exploration documentation of the Dominican Church of Vác, 1994-1995, Tragor Ignác Múzeum. (TIM ND.95.3.1. Munkanapló; ND.95.3.2. 1-262. Feltárási adatlapok).

Fletcher HA, Donoghue HD, Holton J, Pap I, Spigelman M (2003) Widespread occurrence of *Mycobacterium tuberculosis* DNA from 18th-19th century Hungarians. AJPA 120(2):144-152.

Flynn JL, Chan J (2001) Tuberculosis: latency and reactivation. Infect Immun 69(7):4195-4201.

Gernaey AM, Minnikin DE, Copley MS, Power JJ, Ahmed AMS, Dixon RA, Roberts CA, Robertson JD, Nolan J, Chamberlain A (1998) Detecting ancient tuberculosis. Internet Archaeol Available: http://intarch.ac.uk/journal/issue5/gernaey_index.html. Accessed: 03 August 2020.

Getahun H, Matteelli A, Chaisson RE, Raviglione M (2015) Latent *Mycobacterium tuberculosis* infection. N Engl J Med 372:2127-2135.

Glaziou P, Floyd, K, Raviglione M (2018) Trends in tuberculosis in the UK. Thorax 73:702-703.

Golden MP, Vikram HR (2005) Extrapulmonary tuberculosis: an overview. Am Fam Physician 72(9):1761-1768.

Gutierrez MC, Brisse S, Brosch R, Fabre M, Omaïs B, Marmiesse M, Supply P, Vincent V (2005) Ancient origin and gene mosaicism of the progenitor of *Mycobacterium tuberculosis*. PloS Pathog 1(1):e5.

Hartmann S, Minnikin DE (1992) Mycobacterial phenolic glycolipids. In Tyman JHP, Ed., Surfactants in Lipid Chemistry: Recent Synthetic, Physical and Biodegradative Studies. Royal Society of Chemistry, Cambridge, UK, 135-158.

Hershkovitz I, Donoghue HD, Minnikin DE, Besra GS, Lee OY-C, Gernaey, AM, Galili E, Eshed V, Greenblatt CL, Lemma E, Bar-Gal GK, Spigelman M (2008) Detection and molecular characterization of 9000-year-old *Mycobacterium tuberculosis* from a Neolithic settlement in the Eastern Mediterranean. PLOS ONE 3(10):e3426..

Hershkovitz I, Greenwald CM, Latimer B, Jellema LM, Wish-Baratz S, Eshed V, Dutour O, Rothschild BM (2002) Serpens endocrania symmetrica (SES): a new term and a possible clue for identifying intrathoracic disease in skeletal populations. AJPA 118(3):201-216.

Horsburgh CR, Rubin RJ (2011) Latent tuberculosis infection in the United States. N Engl J Med 364:1441-1448.

Kajdocsi Lovász G (2015) A török hódoltság kori idegen etnikumok összehasonlító embertani vizsgálata. PhD Thesis, University of Szeged, Szeged, Hungary.

Kay GL, Sergeant MJ, Zhou Z, Chan JZ-M, Millard A, Quick J, Szikossy I, Pap I, Spigelman M, Loman NJ, Achtman M, Donoghue HD, Pallen MJ (2015) Eighteenth-century ge-

nomes show that mixed infections were common at time of peak tuberculosis in Europe. Nat Commun 6:6717.

Kustár Á, Pap I, Végvári Zs, Kristóf LA, Pálfi Gy, Karlinger K, Kovács KB, Szikossy I (2011a) Using of 3D virtual reconstruction for pathological investigation and facial reconstruction of an 18th century mummified nun from Hungary. In Gill-Frerking H, Rosendahl W, Zink A, Piombino-Mascali D, Eds., Yearbook of Mummy Studies. Verlag Dr. Friedrich Pfeil, München, Germany, 1:83-93.

Kustár Á, Pap I, Végvári Zs, Kristóf LA, Pálfi Gy, Karlinger K, Kovács B, Szikossy I (2011b) Tauber Antónia, 18. századi váci apáca múmiájának patológiai vizsgálata és arcrekonstrukciója 3D rekonstrukciós módszerek alkalmazásával. Anthropol Közl 52:5-15.

Lalremruata A, Ball M, Bianucci R, Welte B, Nerlich AG, Kun JFJ, Pusch CM (2013) Molecular identification of *Falciparum malaria* and human tuberculosis co-infection in mummies from the Fayum Depression (Lower Egypt). PLOS ONE 8(4):e60307.

Lee OY-C, Wu HHT, Donoghue HD, Spigelman M, Greenblatt CL, Bull ID, Rothschild BM, Martin LD, Minnikin DE, Besra GS (2012) *Mycobacterium tuberculosis* complex lipid virulence factors preserved in the 17,000-year-old skeleton of an extinct bison, *Bison antiquus*. PLOS ONE 7:e41923.

Loddenkemper R, Murray JF, Gradmann C, Hopewell PC, Kato-Maeda M (2018) History of tuberculosis. In Migliori GB, Bothamley G, Duarte R, Rendon A, Eds., Tuberculosis. Charlesworth Press, Wakefield, UK, 8-27.

Lönnroth K, Jaramillo E, Williams BG, Dye C, Raviglione M (2009) Drivers of tuberculosis epidemics: The role of risk factors and social determinants. Soc Sci Med 68:2240-2246.

Luna LH, Aranda CM, Santos AL, Donoghue HD, Lee OY-C, Wu HHT, Besra GS, Minnikin DE, Llewellyn G, Williams CM, Ratto N (2020) Oldest evidence of tuberculosis in Argentina: A multidisciplinary investigation in an adult male skeleton from Saujil, Tinogasta, Catamarca (905-1030 CE). Tuberculosis 125:101995.

Maczel M. (2003) "On the traces of tuberculosis" Diagnostic criteria of tuberculosis affection of the human skeleton and their application in Hungarian and French anthropological series. PhD Thesis, University of La Méditerranée, Marseille, France, University of Szeged, Szeged, Hungary.

Marcsik A, Molnár E, Ősz B, Donoghue HD, Zink A, Pálfi Gy (2009) Adatok a lepra, tuberculosis és syphilis magyarországi paleopatológiájához. Folia Anthropologica 8:5-34.

Marcsik A, Szentgyörgyi R, Gyetvai A, Finnegan M, Pálfi Gy (1999) Probable Pott's paraplegia from the 7th-8th century AD. In Pálfi Gy, Dutour O, Deák J, Hutás I, Eds., Tuberculosis: Past and Present. TB Foundation: Szeged, Hungary & Golden Book Publisher: Budapest, Hungary, 331-336.

Masson M, Bereczki Zs, Molnár E, Donoghue HD, Minnikin DE, Lee OY-C, Wu HHT, Besra GS, Bull ID, Pálfi Gy (2015) 7000-year-old tuberculosis cases from Hungary – Osteological and biomolecular evidence. Tuberculosis 95(Suppl. 1):S13-S17.

Matos V, Santos AL (2006) On the trail of pulmonary tuberculosis based on rib lesions: results from the human identified skeletal collection from the Museu Bocage (Lisbon, Portugal). AJPA 130(2):190-200.

Minnikin DE, Besra GS, Lee OY-C, Spigelman M, Donoghue HD (2011) The interplay of DNA and lipid biomarkers in the detection of tuberculosis and leprosy in mummies and other skeletal remains. In Gill-Frerking H, Rosendahl W, Zink A, Piombino-Mascali D, eds., Yearbook of Mummy Studies. Verlag Dr. Friedrich Pfeil, München, Germany, 1:109-114.

Minnikin DE, Bolton RC, Hartmann S, Besra GS, Jenkins PA, Mallet AI, Wilkins E, Lawson AM, Ridell M (1993) An integrated procedure for the direct detection of characteristic lipids in tuberculosis patients. Ann Soc Belg Med Trop 73(Suppl. 1):13-24.

Minnikin DE, Dobson G, Goodfellow M, Magnusson M, Ridell M (1985) Distribution of some mycobacterial waxes based on the phthiocerol family. J Gen Microbiol 131(6):1375-1381.

Minnikin DE, Goodfellow M (1980) Lipid composition in the classification and identification of acid-fast bacteria. In Goodfellow M, Board RG, Eds., Microbiological Classification and Identification. Academic Press, London, UK, 189-256.

Minnikin DE, Lee OY-C, Wu HHT, Besra GS, Bhatt A, Nataraj V, Rothschild BM, Spigelman M, Donoghue HD (2015a) Ancient mycobacterial lipids: Key reference biomarkers in charting the evolution of tuberculosis. Tuberculosis 95(Suppl. 1):S133-139.

Minnikin DE, Lee OY-C, Wu HHT, Nataraj V, Donoghue HD, Ridell M, Watanabe M, Alderwick L, Bhatt A, Besra GS (2015b) Pathophysiological implications of cell envelope structure of *Mycobacterium tuberculosis* and related taxa. In Ribon W, Ed., Tuberculosis – Expanding Knowledge. InTech - Open Access Publisher. 145-75.

Minnikin, DE (1982) Lipids: complex lipids, their chemistry, biosynthesis and role. In Ratledge C, Stanford J, Eds., The Biology of Mycobacteria. Academic Press, London, UK, 95-184.

Molnár E, Donoghue HD, Lee OY-C, Wu HHT, Besra GS, Minnikin DE, Bull ID, Llewellyn G, Williams CM, Spekker O, Pálfi Gy (2015) Morphological and biomolecular evidence for tuberculosis in 8th century AD skeletons from Bélmegyer-Csömöki domb, Hungary. Tuberculosis 95(Suppl. 1):S35-S41.

Niemann S, Kubica T, Bange FC, Adjei O, Browne EN, Chinbuah MA, Diel R, Gyapong J, Horstmann RD, Joloba ML, Meyer CG, Mugerwa RD, Okwera A, Osei I, Owusu-Darbo E, Schwander SK, Rüsh-Gerdes S (2004) The species *Mycobacterium africanum* in the light of new molecular markers. J Clin Microbiol 42(9):3958-3962.

Ortner DJ (2003) Infectious diseases: tuberculosis and leprosy. In Ortner DJ, Ed., Identification of pathological conditions in human skeletal remains. Academic Press, San Diego, CA, USA, 227-271.

Paja L, Coqueugniot H, Dutour O, Willmon R, Farkas GyL, Palkó A, Pálfi Gy (2015) Knee ankyloses associated with tuberculosis from the Medieval Hungary – Differential diagnosis based on medical imaging techniques. Int J Osteoarchaeol 25(3):352-360.

Pálfi Gy, Bereczki Zs, Ortner DJ, Dutour O (2012) Juvenile cases of skeletal tuberculosis from the Terry Anatomical Collection (Smithsonian Institution, Washington, D.C., USA). Acta Biol Szeged 56(1):1-12.

Pálfi Gy, Dutour O, Perrin P, Sola C, Zink A (2015) Tuberculosis in evolution. Tuberculosis 95(Suppl. 1):S1-S3.

Pálfi Gy, Marcsik A (1999) Palaeoepidemiological data of tuberculosis in Hungary. In Pálfi Gy, Dutour O, Deák J, Hutás I, Eds., Tuberculosis: Past and Present. TB Foundation: Szeged, Hungary & Golden Book Publisher: Budapest, Hungary, 531-540.

Pálfi Gy, Molnár E (2009) The paleopathology of specific infectious diseases from Southeastern Hungary: a brief overview. Acta Biol Szeged 53(2):111-116.

Pap I, Józsa L, Repa I, Bajzik G, Lakhani SR, Donoghue HD, Spigelman M. (1999) 18-19th century tuberculosis in naturally mummified individuals (Vác, Hungary). In Pálfi Gy, Dutour O, Deák J, Hutás I, Eds., Tuberculosis: Past and Present. TB Foundation: Szeged, Hungary & Golden Book Publisher: Budapest, Hungary, 419-428.

Pap I, Pálfi Gy, Molnár E, Karlinger K, Kovács KB, Korom Cs, Schultz M, Schmidt-Schultz TH, Spigelman M, Donoghue HD, Kustár Á, Szikossy I (2017) A tuberkulózis előfordulása egy XVIII. századi váci családban. Anthropol Közlem 58:37-47.

Piombino-Mascali D, Jankauskas R, Tamošiūnas A, Valančius R, Gill-Frerking H, Spigelman M, Panzer S (2015) Evidence of probable tuberculosis in Lithuanian mummies. HOMO 66(5):420-431.

Redman JE, Shaw MJ, Mallet AI, Santos AL, Roberts CA, Gernaey AM, Minnikin DE (2009) Mycocerosic acid biomarkers for the diagnosis of tuberculosis in the Coimbra skeletal collection. Tuberculosis 89(4):267-277.

Riojas MA, McGough KJ, Rider-Riojas C, Rastogi N, Hazbón MH (2018) Phylogenomic analysis of the species of the *Mycobacterium tuberculosis* complex demonstrates that *Mycobacterium africanum, Mycobacterium bovis, Mycobacterium caprae, Mycobacterium microti* and *Mycobacterium pinnipedii* are later heterotypic synonyms of *Mycobacterium tuberculosis*. Int J Syst Evol Microbiol 68(1):324-332.

Roberts C (2020) Fashionable but debilitating diseases: Tuberculosis past and present. In Sheridan SG, Gregoricka LA, Eds., Purposeful Pain: The Bioarchaeology of Intentional Suffering. Springer Nature Switzerland AG, Cham, Switzerland, 21-38.

Roberts CA, Lucy D, Manchester K (1994) Inflammatory lesions of ribs: an analysis of the Terry Collection. AJPA 95(2):169-182.

Rock RB, Olin M, Baker CA, Molitor TW, Peterson PK (2008) Central nervous system tuberculosis: pathogenesis and clinical aspects. Clin Microbiol Rev 21(2):243-261.

Rodriguez-Takeuchi SK, Renjifo ME, Medina FJ (2019) Extrapulmonary Tuberculosis: Pathophysiology and imaging findings. RadioGraphics 39(7):2023-2037.

Salo WL, Aufderheide AC, Buikstra J, Holcomb TA (1994) Identification of *Mycobacterium tuberculosis* DNA in a pre-Columbian Peruvian mummy. Proc Natl Acad Sci 91:2091-2094.

Santos AL, Roberts CA (2001) A picture of tuberculosis in young Portuguese people in the early 20th century: a multidisciplinary study of the skeletal and historical evidence. AJPA 115(1):38-49.

Santos AL, Roberts CA (2006) Anatomy of a serial killer: differential diagnosis of tuberculosis based on rib lesions of adult individuals from the Coimbra Identified Skeletal Collection, Portugal. AJPA 130(1):38-49.

Schultz M (1993) Spuren unspezifischer Entzündungen an prähistorischen und historischen Schädeln. Ein Beitrag zur Paläopathologie. Anthropologisches Forschungsinstitut, Aesch, Switzerland & Anthropologische Gesellschaft, Basel, Switzerland.

Schultz M (1999) The role of tuberculosis in infancy and childhood in prehistoric and historic populations. In Pálfi Gy, Dutour O, Deák J, Hutás I, Eds., Tuberculosis: Past and Present. TB Foundation: Szeged, Hungary & Golden Book Publisher: Budapest, Hungary, 503-507.

Schultz M (2001) Paleohistopathology of bone: a new approach to the study of ancient diseases. AJPA 116(Suppl. 33):106-147.

Schultz M (2003) Light microscopic analysis in skeletal paleopathology. In Ortner DJ, Ed., Identification of Pathological Conditions in Human Skeletal Remains. Academic Press, San Diego, CA, USA, 73-107.

Schultz M, Schmidt-Schultz TH (2015) Is it possible to diagnose TB in ancient bone using microscopy? Tuberculosis 95(Suppl. 1):S80-S86.

Spekker O (2018) Evaluation of endocranial bony changes in relation to tuberculosis in the Robert J. Terry Anatomical Skeletal Collection (Washington, DC, USA). PhD Thesis. University of Szeged, Szeged, Hungary.

Spekker O, Hunt DR, Paja L, Molnár E, Pálfi G, Schultz

M (2020a) Tracking down the white plague: The skeletal evidence of tuberculous meningitis in the Robert J. Terry Anatomical Skeletal Collection. PLOS ONE 15(3):e0230418.

Spekker O, Hunt DR, Váradi OA, Berthon W, Molnár E, Pálfi Gy (2018) Rare manifestations of spinal tuberculosis in the Robert J. Terry Anatomical Skeletal Collection (National Museum of Natural History, Smithsonian Institution, Washington, DC, USA). Int J Osteoarchaeol 28:343-353.

Spekker O, Pálfi Gy, Kozocsay G, Pósa A, Bereczki Zs, Molnár E (2012) New cases of probable skeletal tuberculosis from the Neolithic period of Hungary – A morphological study. Acta Biol Szeged 56(2):115-123.

Spekker O, Schultz M, Paja L, Váradi OA, Molnár E, Pálfi G, Hunt DR (2020b) Tracking down the White Plague. Chapter two: The role of endocranial abnormal blood vessel impressions and periosteal appositions in the paleopathological diagnosis of tuberculous meningitis. PLOS ONE 15(9):e0238444.

Spigelman M, Lemma E (1993) The use of the polymerase chain reaction (PCR) to detect *Mycobacterium tuberculosis* in ancient skeletons. Int J Osteoarchaeol 3(2):137-143.

Sreevatsan S, Pan X, Stockbauer KE, Conell ND, Kreiswirth BN, Whittam TS, Musser JM (1997) Restricted structural gene polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionarily recent global dissemination. PNAS 94(18):9869-9874.

Stucki D, Brites D, Jeljeli L, Coscolla M, Liu Q, Trauner A, Fenner L, Rutaihwa L, Borrell S, Luo T, Gao Q, Kato-Maeda M, Ballif M, Egger M, Macedo R, Mardassi H, Moreno M, Vilanove GV, Fyfe J, Globan M, Thomas J, Jamieson F, Guthrie JL, Asante-Poku A, Yeboah-Manu D, Wampande E, Ssengooba W, Joloba M, Boom WH, Basu I, Bower J, Saraiva M, Vaconcellos SEG, Suffys P, Koch A, Wilkinson R, Gail-Bekker L, Malla B, Ley SD, Beck H-P, de Jong BC, Toit K, Sancher-Padilla E, Bonnet M, Gil-Brusola A, Frank M, Beng VNP, Eisenack K, Alani E, Ndung'u PW, Revathi G, Gehre F, Akter S, Ntoumi F, Stewart-Ischerwood L, Ntinginya NE, Rachow A, Hoelscher M, Cirillo DM, Skenders G, Hoffner S, Bakonyte D, Stakenas P, Diel R, Crudu V, Moldovan O, Al-Hajoj S, Otero L, Barletta D, Carter EJ, Diero L, Supply P, Comas I, Niemann S, Gagneux S (2016) *Mycobacterium tuberculosis* lineage 4 comprises globally distributed and geographically restricted sublineages. Nat Genet 48(12):1535-1543.

Szikossy I (2020) Sebészeti beavatkozások nyomai a XVIII. századi váci múmiákon. PhD Thesis. University of Szeged, Szeged, Hungary.

Szikossy I, Bernert Zs, Pap I (1997) Anthropological investigation of the 18th-19th century ossuary of the Dominican Church, Vác, Hungary. Acta Biol Szeged 42:145-150.

Szikossy I, Pálfi Gy, Molnár E, Karlinger K, Balázs K, Kovács KB, Korom Cs, Schultz M, Tyede HS-S, Spigelman M, Donoghue HD, Kustár Á, Pap I (2015) Two positive tuberculosis cases in the late Nigrovits family, 18th century, Vác, Hungary. Tuberculosis 95(Suppl. 1):S69-72.

Váradi OA, Rakk D, Spekker O, Terhes G, Urbán E, Berthon W, Pap I, Szikossy I, Maixner F, Zink A, Vágvölgyi Cs, Donoghue HD, Minnikin DE, Szekeres A, Pálfi Gy (2021) Verification of tuberculosis infection among Vác mummies (18th century CE, Hungary) based on lipid biomarker profiling with a new HPLC-HESI-MS approach. Tuberculosis 126:102037.

Vidal R, Miravitlles M, Caylà JA, Torella M, de Gracia J, Morell F (1997) Increased risk of tuberculosis transmission in families with microepidemics. Eur Respir J 10:1327-1331.

Vuorinen HS (1999) The tuberculosis epidemic in Finland from the 18th to the 20th century. In Pálfi Gy, Dutour O, Deák J, Hutás I, Eds., Tuberculosis: Past and Present. TB Foundation: Szeged, Hungary & Golden Book Publisher: Budapest, Hungary, 105-112.

Wang PD, Lin RS (2000) Tuberculosis transmission in the family. J Infect 41:249-251.

Watanabe M, Aoyagi Y, Ridell M, Minnikin DE (2001) Separation and characterization of individual mycolic acids in representative mycobacteria. Microbiology 147:1825-1837.

World Health Organization. (2020) TB disease burden. In Global Tuberculosis Report 2020. WHO, Geneva, Italy, 23-70.

Zink AR, Sola C, Reischl U, Grabner W, Rastogi N, Wolf H, Nerlich AG (2003) Characterization of *Mycobacterium tuberculosis* complex DNAs from Egyptian mummies by spoligotyping. J Clin Microbiol 41(1):359-367.