



## Tuberculosis in Late Neolithic–Early Copper Age human skeletal remains from Hungary



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### S U M M A R Y

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Alsónyék–Bátaszék in Southern Hungary is one of the largest late Neolithic settlements and cemeteries excavated in Central Europe. In total, 2359 burials from the Late Neolithic – Early Copper Age Lengyel culture were found between 2006 and 2009 [1]. Anthropological investigations previously carried out on individuals from this site revealed an interesting paleopathological case of tuberculosis in the form of Pott's disease dated to the early 5<sup>th</sup> millennium BC. In this study, selected specimens from this osteo-archaeological series were subjected to paleomicrobiological analysis to establish the presence of MTBC bacteria. As all individuals showing clear osteological signs of TB infection belonged to a single grave group, 38 individuals from this grave group were analysed. The sample included the case of Pott's disease as well as individuals both with and without osseous TB manifestations. The detection of TB DNA in the individual with Pott's disease provided further evidence for the occurrence of TB in Neolithic populations of Europe. Moreover, our molecular analysis indicated that several other individuals of the same grave group were also infected with TB, opening the possibility for further analyses of this unique Neolithic skeletal series.

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### 1. Introduction

Tuberculosis (TB) is one of the oldest diseases and still has a high prevalence nowadays. The disease is pandemic, with 2.5 billion people on Earth infected by bacteria of the genus *Mycobacterium* [2]. The main human pathogens in this genus causing tuberculosis are *Mycobacterium tuberculosis*, *Mycobacterium bovis* and *Mycobacterium africanum*, which belong together with *Mycobacterium microti*, *Mycobacterium canetti*, *Mycobacterium caprae* and *Mycobacterium pinnipedii* to the so-called *M. tuberculosis* complex [3].

Members of *M. tuberculosis* complex (MTBC) derived from a common ancestor, *Mycobacterium protuberulosis*, which evolved together with mankind [4–6]. Socio- and economic changes taking place during the Neolithic, including settlements, crop cultivation and animal domestication, led to a much closer contacts between humans and animals. These new living conditions for humans during Neolithic facilitated the transmission and spread of infectious diseases like TB between humans but also between humans and animals. The latter transmission pathway led to the long-lasting theory that *M. tuberculosis* evolved from *M. bovis* by transmission from cattle to humans during domestication [7]. Several studies, however, in particular genome sequencing, contradict this “bovis to human” scenario by confirming that MTBC complex strains have undergone reductive evolution starting with an ancient form of *M. tuberculosis* which developed into modern *M. tuberculosis*

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strains, i.e., *M. africanum* and, finally, different *M. bovis* strains [8–10].

In addition the modern genetic analysis of TB specific skeletal lesions in ancient human remains helps to better understand the occurrence and spread of this devastating disease during important periods such as the Neolithic. Linking macromorphological investigation with ancient DNA and lipid biomarker based TB diagnostic provided the earliest biomolecular evidence of tuberculosis in humans at the Early Neolithic site of Atlit-Yam, Israel, dated to 9250–8160 BP [11]. The oldest published paleopathological and molecular TB cases in Europe come from Neolithic Hungary [12] and Germany [13]. The latter study in particular provided important insights into the health status of Early Neolithic populations in Central Germany by analysing several human skeletal remains of three different sites.

In this study, we provided additional paleopathological and molecular evidence of the occurrence of TB in Europe during the Neolithic. TB infection in an individual with Pott's gibbus was molecularly confirmed. Moreover, our molecular analysis indicated that several other individuals from the same grave group in the Alsónyék–Bátaszék site were infected with TB, opening the possibility for further analyses of this unique Neolithic osteological series.

## 2. Material and methods

### 2.1. The osteological series

Alsónyék–Bátaszék is situated in Southern Hungary. The excavation of this prehistoric settlement and cemetery took place between 2006 and 2009, during which 2359 burials of the Late Neolithic-Early Copper Age Lengyel culture were found (first half of the 5th millennium BC) [1,14,15]. This is to date one of the largest late Neolithic site excavated in Central Europe. The first TB cases were uncovered from the northern, so-called Kanizsa-dűlő part (or O10/B) of the site, from where the skeletal remains of 862 graves were excavated (Figure 1) [16].



Figure 2. Severe case of Pott's disease from Alsónyék–Bátaszék, grave no.4027.

The graves were usually arranged in smaller or larger groups, which have been assumed to correlate with familial relationships between the deceased. The deceased were laid with their side either to the East or to the West, their face to the South and in a crouched position, the typical burial position for this period. During the anthropological examination of this series, Köhler and her colleagues detected an interesting case of Pott's disease in grave no. 4027 (Figure 2), which belonged to grave group 13 (Figure 1) [1]. The individual clearly showed osteological manifestations of tuberculosis. Since this case belonged to a grave group, the skeletal remains of the other 38 individuals buried in the same group were also macromorphologically investigated, with a particular focus on typical osteological manifestations as well as early-stage/atypical skeletal lesions of the disease. In addition, due to the relatively good state of preservation of the material and the importance of the chronological period of this series, ancient DNA analysis to diagnose TB DNA in the skeletal material was performed on individuals of this grave group.

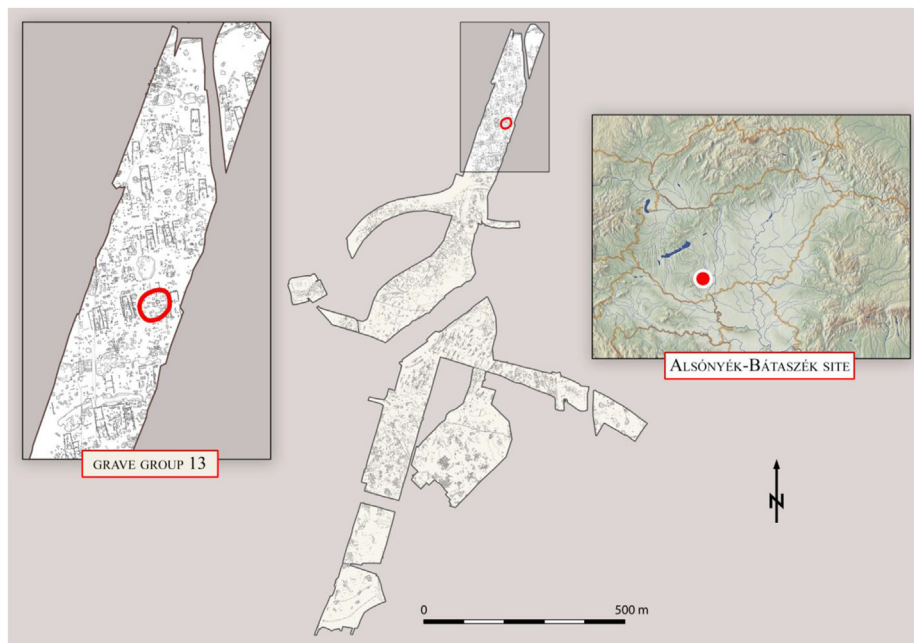


Figure 1. Overview map of the Neolithic site of Alsónyék–Bátaszék in Southern Hungary. The grave group 13 used in this study is highlighted with a red circle. (For interpretation of the references to colour in this figure caption, the reader is referred to the web version of this article.)

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