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Letter to the Editor

Detection of *Mycobacterium leprae* DNA from remains of a medieval individual, Amiens, France

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To the Editor,

Leprosy, caused by the bacterial pathogen *Mycobacterium leprae*, has been observed in humans since remote times. Ancient Sanskrit texts written as early as 600 BC describe both nervous and skin damage [1]. Skeletal changes, i.e. specific and non-specific bone alterations, are observed in ~5% of individuals with leprosy [1,2]; the oldest evidence of the disease in ancient bones can be traced back to c.3500 BC in Hungary [3].

Initial molecular identification of the pathogen on archaeological specimens was described in 1993. Additional knowledge on *M. leprae* from ancient sources was provided by subsequent PCR-based and whole-genome-sequencing approaches [1,4,5].

Despite several studies on the repartition of leprosy and the different phylogenetic branch of *M. leprae* among European populations [6], detection and characterization of this pathogen on ancient samples from medieval France has not been published.

We studied one individual from the urban cemetery of Caudron Street (Amiens city, northern France (see [Supplementary material, Appendix S1](#)). This skeleton, SP20076, fairly incomplete and dated from the tenth to eleventh centuries, displays skeletal lesions suggestive of leprosy, according to the palaeopathological literature ([Fig. 1a](#); see [Supplementary material, Appendix S2 and Fig. S1](#)). The

investigated individual is an adult of unknown sex, represented only partially by the lower limb elements.

A right lateral cuneiform bone and a right navicular bone were collected and fully documented (in descriptions and pictures) before proceeding to molecular investigation. One hundred milligrams of bone powder was used from each sample for DNA extraction (NucleoSpin DNA Trace, Macherey-Nagel, Hoerd, France).

PCR amplification systems used for detection and genotyping of *M. leprae* DNA were performed using RLEP, REPLEP, ML1545 and 18-kDa antigen target sequences, whereas four single nucleotide polymorphisms (SNPs) were investigated for strain genotyping, i.e. SNPs 14 676, 1 642 875, 2 935 685 and 1 133 492 (see [Supplementary material, Table S1](#)). Nucleic acid extracts were also tested for the presence of *Mycobacterium tuberculosis* complex DNA using PCR detection systems for IS6110 and IS1081 sequences (see [Supplementary material, Table S2](#)). PCR products were cloned and analysed by Sanger sequencing (Eurofins GATC Biotech, Constance, Germany). Sequence analysis was performed using NCBI's online Basic Local Alignment Search Tool (BLAST) and the GenBank database; nucleotide alignments were performed using MEGA7 (<https://www.megasoftware.net/>).

Initial investigation of *M. leprae* DNA using RLEP, REPLEP, ML1545 and 18-kDa PCR systems generated amplification products of the expected sizes for both sample extracts studied at dilutions 1:20 and 1:50; no signal was obtained for undiluted extracts and negative controls. Subsequent sequence comparisons with those deposited in databases confirmed the systematic *M. leprae* origin of amplified DNA fragments (see [Supplementary material, Fig. S2a](#)); no sequence variation was observed between cloned amplicons.

Successful amplification and sequencing results were also obtained for the four targeted SNPs sequences (see [Supplementary material, Fig. S2b](#)). Based on the precise analysis of the three main loci described by Monot et al. [5], informative positions for SNPs 14 676, 1 642 875 and 2 935 685 corresponded to C/T/C bases, respectively: accordingly, the SP20076 case was identified as SNP type 3 ([Fig. 1b](#)). Further genotyping information was deduced from the T/G polymorphism at nucleotide position 1 133 492: the identification of a T base showed the strain of leprosy belonged to the

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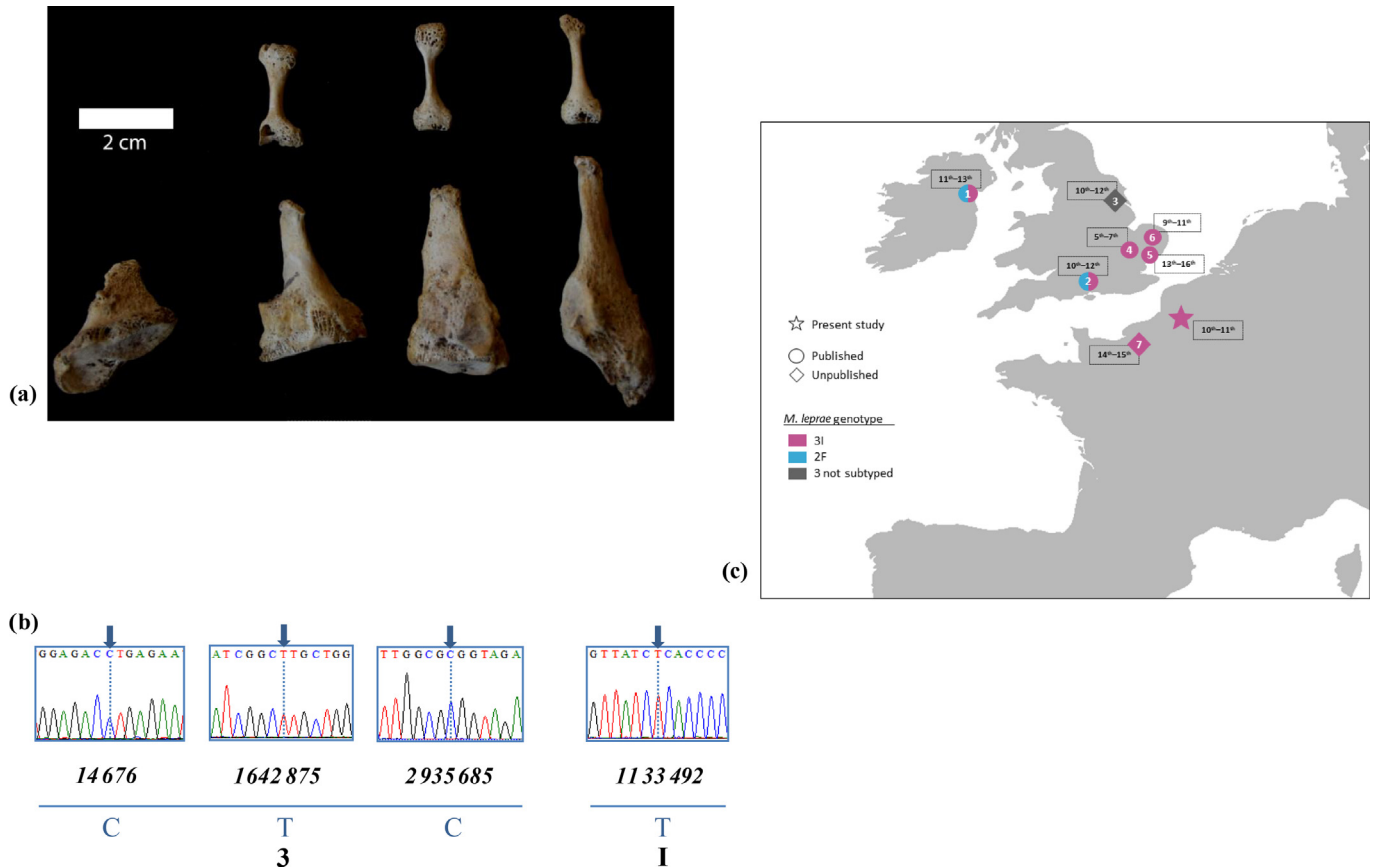


Fig. 1. (a) SP20076 case: anterior view of the right foot bones; (b) SP20076 *M. leprae* genotype 3I deduced from phylogenetically informative loci investigated. SNPs partial sequences and informative positions (arrow) are shown; (c) Map summarizing archeological cases in medieval Britain, France and Ireland with *M. leprae* identification and genotyping. Symbol n°: 1, Taylor et al. [6]; 2, Taylor et al. [8]; 3, Unpublished; 4, Inskip et al. [9]; 5, Taylor et al. [10]; 6, Inskip et al. [11]; 7, Donoghue et al. [7].

3I lineage according to the typing system adopted currently [5] (Fig. 1b).

SP20076 aDNA results were identical following a second round of nucleic acids extraction/molecular detection procedures. No evidence of *M. tuberculosis* complex DNA was found in the samples studied.

Strain genotyping, using a well-established typing system based on SNP analysis, permitted us to deduce the presence of an *M. leprae* 3I strain in the investigated human remains; reproducible and systematic amplifications of the multiple target sequences considered in the study reinforced this diagnosis.

A high genetic diversity of *M. leprae* in medieval European regions is demonstrated by the distinct phylogenetic branches in ancient samples, with the general preponderance of 2F and 3, but also 4 and 0 [4,6]. Notably, branch 3 (incorporating 3I strains) was present in Europe from at least the fifth century AD [4]. *Mycobacterium leprae* 3I strains were retrieved in human remains dated from the tenth to eleventh centuries AD (this study) and the fourteenth to fifteenth centuries AD [6,7], originating from two sites in northern France (Amiens and Saint-Thomas d'Aizier, respectively), which are about 120 km apart (Fig. 1c). This underlines the presence and persistence of *M. leprae* branch 3 in medieval France, or at least its northern part. These findings, in association with the identification of *M. leprae* branch 3 in human remains identified in Britain, Ireland, Denmark and Sweden, indicate a predominant presence of this lineage in northern medieval Europe [4,6].

Studies in French human remains from distinct periods and locations should be performed; such approaches, associated with the

exploration of new European/non-European sites, will contribute to a better knowledge of the dissemination of *M. leprae* in ancient populations.

Transparency declaration

The authors declare that there are no conflicts of interest. The work was supported by the UMR 7268 ADES, the French Blood Agency PACA Corse and the French Ministry of Research (no specific grant).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2019.09.011>.

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