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A migration-driven model for the historical spread of leprosy in medieval Eastern and Central Europe

Helen D. Donoghue [a,*], G. Michael Taylor [b], Antónia Marcsik [c], Erika Molnár [d], Gyorgy Pálfi [d], Ildikó Pap [e], Maria Teschler-Nicola [f], Ron Pinhasi [g], Yilmaz S. Erdal [h], Petr Velemínsky [i], Jakub Likovsky [j], Maria Giovanna Belcastro [k,l], Valentina Mariotti [k,m], Alessandro Riga [k], Mauro Rubini [n,o], Paola Zaio [n], Gurdyal S. Besra [p], Oona Y.-C. Lee [p], Houdini H.T. Wu [p], David E. Minnikin [p], Ian D. Bull [q], Justin O'Grady [a,1], Mark Spigelman [a,r]

[a] Centre for Clinical Microbiology, Division of Infection and Immunity, University College London, UK
[b] Department of Microbial and Cellular Science, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK
[c] University of Szeged, Mályva utca 23, H-6771 Szeged, Hungary
[d] Department of Biological Anthropology, University of Szeged, Hungary
[e] Department of Anthropology, Natural History Museum, Budapest, Hungary
[f] Department of Anthropology, Natural History Museum, Vienna, Austria
[g] School of Archaeology and Earth Institute, Belfield, University College Dublin, Dublin 4, Ireland
[h] Department of Anthropology, Hacettepe University, Ankara, Turkey
[i] Department of Anthropology, National Museum, Prague, Czech Republic
[j] Department of the Archaeology of Landscape and Archaeobiology, Institute of Archaeology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[k] Laboratorio di Bioarcheologia e Osteologia Forense, Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Via Selmi 3, 40126 Bologna, Italy
[l] Centro Fermi, Piazza del Viminale 1, 00184 Rome, Italy
[m] ADES, UMR 7268 CNRS/Université de la Méditerranée/EFS, Université de la Méditerranée, CS80011, Bd Pierre Dramard,13344 Marseille Cedex 15, France
[n] Department of Archaeology, Foggia University, Tivoli, Italy
[o] Anthropological Service of S.B.A.L. (Ministry of Culture), Rome, Italy
[p] Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Edgbaston, Birmingham, UK
[q] Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol, UK
[r] Department of Anatomy and Anthropology Sackler Medical School, Tel Aviv University, Israel

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ABSTRACT

Leprosy was rare in Europe during the Roman period, yet its prevalence increased dramatically in medieval times. We examined human remains, with paleopathological lesions indicative of leprosy, dated to the 6th–11th century AD, from Central and Eastern Europe and Byzantine Anatolia. Analysis of ancient DNA and bacterial cell wall lipid biomarkers revealed *Mycobacterium leprae* in skeletal remains from 6th–8th century Northern Italy, 7th–11th century Hungary, 8th–9th century Austria, the Slavic Greater Moravian Empire of the 9th–10th century and 8th–10th century Byzantine samples from Northern Anatolia. These data were analyzed alongside findings published by others. *M. leprae* is an obligate human pathogen that has undergone an evolutionary bottleneck followed by clonal expansion. Therefore *M. leprae* genotypes and sub-genotypes give information about the human populations they have infected and their migration. Although data are limited, genotyping demonstrates that historical *M. leprae* from Byzantine Anatolia, Eastern and Central Europe resembles modern strains in Asia Minor rather than the recently characterized historical strains from North West Europe. The westward migration of peoples from Central Asia in the first millennium may have introduced different *M. leprae* strains into medieval Europe and certainly would have facilitated the spread of any existing leprosy. The subsequent decline of *M. leprae* in Europe may be due to increased host resistance. However, molecular evidence of historical leprosy and tuberculosis co-infections suggests that death from tuberculosis in leprosy patients was also a factor.

1. Introduction

Leprosy (Hansen's Disease) is primarily a disease of peripheral nerves and skin but also affects bones. In the multi-bacillary lepro-

* Corresponding author at: Centre for Clinical Microbiology, Division of Infection and Immunity, University College London, London NW3 2PF, UK. Tel.: +44 (0)207 7940500x31146.
E-mail address: h.donoghue@ucl.ac.uk (H.D. Donoghue).
[1] Present address: Norwich Medical School, University of East Anglia, Norwich, UK.

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matous state there is direct invasion of soft tissues around the face and mouth by *Mycobacterium leprae* and spread via the peripheral nerves to the long bones and extremities. These changes in physical characteristics enabled the disease to be recognised in antiquity (Skinsnes and Chang, 1985). Although diagnoses based only on written reports remain questionable, they suggest that leprosy existed in ancient times in Egypt, India and China (Lechat, 1999; Mark, 2002). There is possible skeletal evidence of leprosy from 2000 BC Rajasthan and the late Indus civilisation from 2500–1700 BC (Robbins Schug et al., 2013). The most diagnostic bone changes are found in the skull, described as the rhinomaxillary syndrome, that involves the destruction of the anterior nasal spine, the rounding and widening of the nasal margins, the partial resorption of the pre-maxillary alveolar process and in some cases the loss of the upper incisors (Møller-Christensen, 1961; Ortner, 2003). Additional changes include deformities of the hands and feet, which are usually symmetrical and involve joint destruction, resorption of the fingers and toes, with potentially partial dislocation and bone fusion (Ortner, 2003).

A major difficulty in diagnosing leprosy in skeletal remains is that syphilis may cause similar changes in the rhinomaxillary region, while psoriatic arthritis, septic arthritis and other joint diseases may cause identical changes in the hands and feet (Ortner and Putschar, 1985). Hence a clear diagnosis of leprosy based solely on paleopathology can be made only if the typical facial changes are found in combination with atrophy and truncation of the fingers and toes. Not all leprosy cases display changes in both the rhinomaxillary region and the hands and feet, making paleopathological diagnosis difficult. Furthermore, as skeletal collections often comprise incomplete and damaged bones, paleopathological diagnosis is likely to overlook many true leprosy cases due to insufficient evidence. As *M. leprae* is an obligate pathogen, its presence in ancient human remains provides clear evidence of infection (Donoghue et al., 2002). Ancient DNA (aDNA) and/or lipid biomarker analyses enable identification of *M. leprae*, thereby confirming the antiquity of the disease. If aDNA preservation is sufficient, phylogenetic data may be obtained, but analysis is often restricted to the confirmation of probable leprosy cases, identified by paleopathological features.

Only about 5% of lepromatous leprosy, diagnosed in the 20th century before the introduction of antibiotics, involved bone changes (Faget and Mayoral, 1944). Therefore, the number of leprosy cases diagnosed by paleopathology will always be an under-estimate. However, comparison of the number of leprosy cases based on paleopathology against the number of skeletons systematically examined for typical lesions for a given period in antiquity, gives a glimpse of changes in the prevalence rates of the disease over space and time. In Britain the earliest evidence of leprosy was found in 2/1480 specimens from Romano-British sites (0.14% prevalence), in 18/2031 specimens from the 5th–11th centuries AD (0.89% prevalence) and in 108/4742 specimens dated from the 12th–16th centuries (2.28% prevalence) (Roberts, 2002). This is consistent with the historical accounts and suggests that the earliest appearance of the disease in Europe occurred during the Roman period (Pinhasi et al., 2006).

A major historic transition occurred when early Eurasian civilizations came into military and commercial contact some 1500–3000 years ago (McMichael, 2001, 2004). The east–west trade route, known as the Silk Road, was a means of spreading infections to and from China, the Eastern Mediterranean and Rome, to previously unexposed populations, including malaria, bubonic plague, leprosy, measles and smallpox. For example, McMichael (2001) states that smallpox entered the Roman Empire via troops returning from Syria in the second century AD. Mark (2002) suggested that the troops of Alexander the Great brought leprosy from eastern Asia to the Mediterranean, leading to its spread on a larger scale in Europe during the fourth century BC.

Skeletal cases with evidence of pathological lesions that are consistent with leprosy were reported from 4th to 3rd century BC Bologna, Italy (Mariotti et al., 2005) and 2nd century BC Roman Egypt (Molto, 2002). A case of lepromatous leprosy from mummified remains in early Christian Nubia (Elliot Smith and Dawson, 1924) and there are several reported cases from the Byzantine period (Zias, 1985). A child with characteristic leprosy paleopathology was found in Martellona (Rome, Central Italy), dated to the 2nd–3rd century AD (Rubini et al., 2012). An adult from Palombara, a poor rural site near Rome, Central Italy, showed paleopathology of the rhinomaxillary region typical of leprosy (Rubini et al., 2014). This case was C^{14}-dated to 475 ± 25 years CE (5th century AD). Among other early cases, Reader (1974) reported changes suggestive of leprosy in the right foot of an incomplete adult skeleton from a 4th century AD Romano-British cemetery. Also, a case from the Roman Iron Age (0–400 AD) has been reported in Sweden (Arcini and Artelius, 1993 cited by Kjellström (2010)). Hence, there is sporadic evidence of leprosy in the 'Roman World' that may have extended west to southern Britain and north to southern Sweden.

The diagnosis of *M. leprae* in specimens using both paleopathological diagnosis and aDNA analysis was first reported by Rafi et al. (1994) in archaeological skeletal samples from early Christian Palestine (600 AD). The earliest case confirmed by aDNA analysis, also from the Eastern Mediterranean, was dated to the 1st century AD (Donoghue et al., 2005a; Matheson et al., 2010). The *M. leprae* genome contains several repetitive sequences that enable the identification of the organism. Single nucleotide polymorphisms (SNPs) form the basis of molecular typing (Monot et al., 2005). There appears to be a clonal relationship between *M. leprae* and its human host, so determination of the genetic profiles of modern and extinct strains of *M. leprae* can illuminate the migration and spread of pathogen and host over time (Monot et al., 2009; Economou et al., 2013; Schuenemann et al., 2013; Taylor et al., 2013; Mendum et al., 2014). Archaeological studies indicate that the first significant appearance of leprosy occurred in northern Europe during the 9th–11th century AD (Schuenemann et al., 2013; Taylor et al., 2013). In Britain, the increase of lazar house foundations for the care of leprosy patients was maximal during the 12th and early 13th centuries (Manchester and Roberts, 1989). During the 15th–16th centuries the disease nearly disappeared from southern Europe and Britain, possibly linked to the increased level of tuberculosis in the community (Manchester, 1984).

Much less is known about the appearance and spread of leprosy in Eastern Europe and Western Asia. The paleopathological study by Blau and Yagodin (2005a) indicates evidence of leprosy from a nomadic burial mound located in the Ustyurt Plateau, Uzbekistan, radiocarbon dated to 80–240 AD (OxA-11792 on human tooth, 2 sigma) (Blau and Yagodin, 2005b). This suggests that leprosy prevailed among nomadic central Asian people and that one or more of these Asian populations may have either introduced leprosy for the first time in Eastern Europe by the 6th–8th century AD, or possibly re-introduced it as a later wave following the Roman period spread of the disease across Europe. However, there is a lack of evidence of leprosy from the skeletal population in the eastern parts of the Roman Empire, such as Croatia, where the earliest historical report of the disease was 804 AD and linked to contact with Byzantium (Bakija-Konsuo and Mulić, 2011).

The movement of peoples from Central Asia into the Great Hungarian Plain (Holló et al., 2008) and Northern Italy (Rubini and Zaio, 2011) may be relevant in relation to the spread of leprosy. Cases recognised by paleopathology were reported from cemeteries in 6th–8th century Central Italy (Belcastro et al., 2005; Rubini and

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Zaio, 2009) and from a 10th century cemetery in Eastern Hungary (Pálfi, 1991). Later, combined paleopathological and aDNA studies of specimens from early medieval sites in Eastern Hungary (Haas et al., 2000; Csóri et al., 2009) and southern Hungary (Donoghue et al., 2005a) confirmed that the disease existed in Eastern Europe during this period.

A key to our understanding of spatio-temporal changes in disease patterns is the identification of new leprosy cases from well-dated archaeological contexts and their differential diagnosis, using both paleopathological and molecular methods (Donoghue et al., 2005a; Minnikin et al., 2011). The present study focuses on the confirmation of leprosy in Northern Anatolia, Eastern and Central Europe, during the 6th–11th centuries AD and the molecular characterisation of *M. leprae*. This was achieved by collating earlier results and systematically assessing the presence of leprosy in eight Avar period cemeteries, six Early Mediaeval sites, and a Byzantine North Anatolian cemetery.

2. Materials and methods

2.1. Paleopathological assessment

Specimens analyzed originated from Austria, the Czech Republic, Hungary, Italy and Turkey, dated from the 6th–8th to the 11th centuries (Table 1). Further details and descriptions of the additional archaeological sites and burials are available as Electronic supplementary material S1. Possible leprosy cases were first identified according to established paleopathological signs (S1 supplementary text and figures) and were assessed using standard macroscopic methods.

2.2. Molecular analysis

Taking strict precautions against contamination (Supplementary material S2), DNA extracts were made for all specimens with pathological conditions consistent with infection by the pathogen. *M. leprae* multi-copy and single-copy loci were amplified by PCR, with independent laboratories to provide verification of data, using established methods for paleomicrobiological analysis (S2 Table 1) based on the repetitive sequences RLEP (Donoghue et al., 2001, 2005a; Taylor et al., 2011) and RepLep. Better-preserved samples were genotyped (Monot et al., 2005) according to three single nucleotide polymorphisms (SNPs) and sub-genotyped, where possible (Monot et al., 2009; Taylor et al., 2006, 2013). Strains were further distinguished by microsatellite analysis (Taylor and Donoghue, 2011; Taylor et al., 2013). *M. leprae* cell wall lipid biomarkers (Supplementary text and figures S3) were used to provide independent confirmation of aDNA findings (Redman et al., 2009; Lee et al., 2012). Several specimens were also examined for the presence of *M. tuberculosis* aDNA (Supplementary text and Table S2).

3. Results

A detailed paleopathological macroscopic analysis of putative leprosy cases, together with an assessment of published cases, identified material for molecular examination (Supplementary text and figures S1). *M. leprae* DNA was found in specimens at all sites, including seven from Hungary, two sites from Italy, and one site each from Austria, the Czech Republic and Turkey (Table 1). The timescale ranged from the 6th–8th to the 11th centuries. In several specimens *M. tuberculosis* aDNA was also detected, confirming earlier findings of such co-infections (Donoghue et al., 2005a).

Genotyping was successfully performed for *M. leprae* DNA (Supplementary text and figures S2) obtained from 7th century Hungary, 8th–9th century Turkey, 9th–10th century Czech Republic and 10th century Hungary. All were of SNP-type 3. This is associated in the modern day with strains from Europe, North Africa, the Far East and the Americas (Monot et al., 2005, 2009; Weng et al., 2013). Where sub-genotyping was possible, the Hungarian and Byzantine samples from the 7th to 10th centuries were of subtype 3 K. Two samples, from 9th to 10th century Czech Republic and 10th century Hungary were of subtype 3M (Table 1).

Two samples from Hungary, from the 10th century, plus one sample from the Czech Republic (9th–10th century), each yielded unique microsatellite data that indicates the excellent state of preservation of these specimens (Taylor and Donoghue, 2011). The *M. leprae* DNA from the two individuals in the adjoined Hungarian burial site of Püspökladány-Eperjesvölgy differed in the number of TTC repeats, having 12 and 17 copies respectively. Interestingly, these two 10th-century *M. leprae* strains were of different sub-genotypes, 3K and 3M. Therefore, different sub-genotypes of *M. leprae* were contemporaneous in the same burial ground. This has also been noted in burial grounds from North West Europe, where individuals with *M. leprae* genotypes 2F or 3I were identified (Economou et al., 2013; Taylor et al., 2013).

M. leprae cell wall lipids appear to be more persistent than aDNA, as shown by the data from 7th century Vicenne, Italy, where no aDNA but lipid biomarkers were found in a well-developed case of leprosy in specimen 144 (Table 1 and Supplementary text and figures S3). Strong, coherent profiles of total mycolic acids were recorded for specimens 18R, 18L and 144, but they differed slightly from the modern *M. leprae* standard (S3 Fig. 1). The profiles of the purified α-mycolates were again coherent, but the overall profiles were essentially four carbons shorter than those from standard *M. leprae* (S3 Fig. 2). In contrast, the more robust mycocerosates showed an excellent profile of C_{30} to C_{34} mycocerosates for specimen 18R (S3 Fig. 3), corresponding closely with the *M. leprae* standard (S3 Fig. 4). The same mycocerosates, typical of *M. leprae*, were seen for 18L and 144, but with much reduced intensity (S3 Fig. 3).

4. Discussion

Geographical analysis shows that regions, apparently endemic for leprosy, are associated with migrations linked with military activity and aggressive expansion of territories, or of colonization (Buzhilova, 2002). Early sporadic reports of leprosy in Western Europe (Manchester, 1984; Lechat, 1999; Brothwell et al., 2000; Blondiaux et al., 2002) are believed to be associated with Roman armies and traders. The premise that leprosy originated in the East and came to Egypt via seaborne trade during this period is discussed by Mark (2002) who thought that this was more likely than the widely-held belief that the disease was brought back by the armies of Alexander the Great (Dols, 1979; Monot et al., 2005). After the fall of the Roman Empire there was an expansion westwards of peoples from Central Asia. The Avars, believed to belong to the Juan–Juan confederation of Mongolian pastoralist tribes, emerged in the 4th century AD (Zhang and Rong, 1998) and spread into Eastern and Central Europe (Fig. 1). Diagnosis of leprosy in 1st–4th century AD Uzbekistan (Blau and Yagodin, 2005a,b), using paleopathology and subsequent molecular analysis (Taylor et al., 2009), demonstrates the antiquity of leprosy in Central Asia and suggests that the disease could have been spread by the Avars, resulting in the appearance of leprosy in Hungary, Eastern Austria and Italy. Another possibility is that the Avars played a role in the spread of indigenous leprosy from Asia Minor to Eastern and Central Europe. Leprosy was a disease known in Byzantium (Dols, 1979) and a gold Byzantine solidus was recovered from Grave 21 in Kiskundorozsma-Daruhalom, Hungary. This represents the latest coin of the series issued under Constans II (Constans II, MIB

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Table 1
Summary of age and geographical location of burial sites, details of specimens examined and molecular biomarkers detected.

Country and site	Burial No.	Age at death	Sex	Samples	Century (AD)	M. leprae DNA	SNP type	M. leprae lipids	MTB[1] DNA
Hungary	79	35–45	M	Rib	11th	+		+	+
Lászlófalva-Szentkirály				Tarsus		+			
Hungary	2467	35–39	F	Nasal	11th	–			+
Felgyő, Kettőshalmi-dűlő	3658	Elderly	M	Tibia		+			
Hungary	11	55–60	F	Nasal	10th	+			
	222	22–40	M	Nasal	10th	+	3K		+
Püspökladány-Eperjesvölgy	429	50–55	M	Nasal	11th	+			
	503	30 –35	F	Nasal	10th	+	3M	+	+
Hungary	S202	50–60	F	Metatarsal	10th	–			
	S237a	Middle-aged	M	Palate	10th	+			
Sárrétudvari-Hízóföld	S237b			Metatarsal		–			
Hungary	HG-56	Mature	M	Palate	10th	+			+
Hajdúdorog-Gyúlás									
Czech Republic	188	12–15	M	Nasal	9th–10th	+	3M		
Prušanky				Fibula		+			
				Arm		+			
				Rib		±			
Austria Zwölfaxing	70	65–70	F	Palate & Rib	8th–9th	+			
	88	25–30	M	Palate & Rib		+			+
Croatia	2A	50–60	M	Rhino-maxillary	8th–9th	+	3		
Radasinovci	3A	20–25	F			+	3		
Watson et al. (2009)									
Turkey	9/1	4–5 m	–	Skull	8th–9th	+			
	11/2	40–50	M	Nasal		–			
Kovuklukaya	20/1	35–45	F	Nasal		+	3K		
	24/1	30–35	M	Nasal		+		+	
Hungary	SG38	30–35	F	Rhino-maxillary	Late 7th–9th	+		+	
Szarvas Grexa									
Hungary	BC-51	23–25	M	Maxilla/Nasal	7th–9th	+			
Bélmegyer-Csömöki domb									
Hungary	SK11	Adult	F	Maxilla/Nasal	7th–8th	+			
Szentes-Kistőke									
Italy	T18	Young adult	F	Nasal	Mid-late 7th	+		+	
				Tibia		+			
Vicenne	T31	Mature	F	Maxilla		–			
	T144	20–25	M	Tibia		–		+	
Hungary	KD271	50–60	M	Palate	7th	+	3K		
Szeged-	KD517	35–40	M	Nasal		+		+	+
Kiskundorozsma-Daruhalom dűlő II	KD518	40–45	M	Nasal		+			
Italy	T68	46–48	F	Maxilla	6th–8th	–			
				Nasal		–			
				Rib		–			
Morrione	T108	50–55	M	Maxilla		+			
				Nasal		–			
				Rib		+			
Uzbekistan	5b	Adult	F	Skull	1st–4th	+	3L	+	
Devkesken 6				Left tibia		+			

[1] MTB, *M. tuberculosis.*

39, AD 667–668). Thus there is circumstantial evidence to suggest that males of this Avar community had contacts with the Byzantine Empire (Donoghue et al., 2005b). This coin was probably minted for about a year, and it is thus reasonable to assume that the journey occurred sometime in the late AD 660's or early AD 670's.

Following their arrival in the Byzantine Empire and southeast Europe, the Avars migrated westwards and clashed with the kingdom of the Franks. There is both historical and archaeological evidence of Avar occupation in the area at this time, and that they formed alliances with local Slavs. The case from the 9th century Czech Republic (Taylor and Donoghue, 2011) is relevant as it is from the Slavic Greater Moravian Empire, known to have fought both alongside and against the Avars, thereby providing means of spread of the disease. The confirmation of several cases of leprosy identified by skeletal paleopathology, reported from 7th to 9th century AD Avar settlements in Hungary (Marcsik et al., 2009; Pálfi and Molnár, 2009), is the earliest report of the disease in this region. Similarly, the finding of *M. leprae* DNA in an 8th–9th century Austrian sample pre-dates all other known cases of leprosy in that country.

Leprosy has also been found in 6th–8th century Italy. The leprosy case from the 7th century cemetery of Vicenne-Campochairo, Italy was from a Barbarian complex with Lombard, local and Asian grave goods (Belcastro et al., 2005). Rubini and Zaio (2009) described two burials with leprosy paleopathology in a cemetery attributed to a semi-nomadic Lombard-Avar group. Both sites may have represented military outposts to control the area against Byzantine invasions. The present biomolecular study confirms *M. leprae* at both sites. The molecular confirmation of *M. leprae* in 8th–9th century Croatia (Watson et al., 2009) is consistent with the earliest historical report of leprosy at this location in 804 AD (Bakija-Konsuo and Mulić, 2011), especially as it is suggested that the disease resulted from contact with Byzantium.

A 4th century sample from the Dakhleh Oasis in Roman Egypt was of genotype 3 (Monot et al., 2005), the same main branch as those linked to the Avar expansion, although it was not possible to determine the sub-genotype. The earliest *M. leprae* to be sub-genotyped, from 1st to 4th century Uzbekistan, was of subtype 3L (Taylor and Donoghue, 2011). It is of interest that the predominant sub-genotype in the earlier Hungarian samples was 3K, iden-

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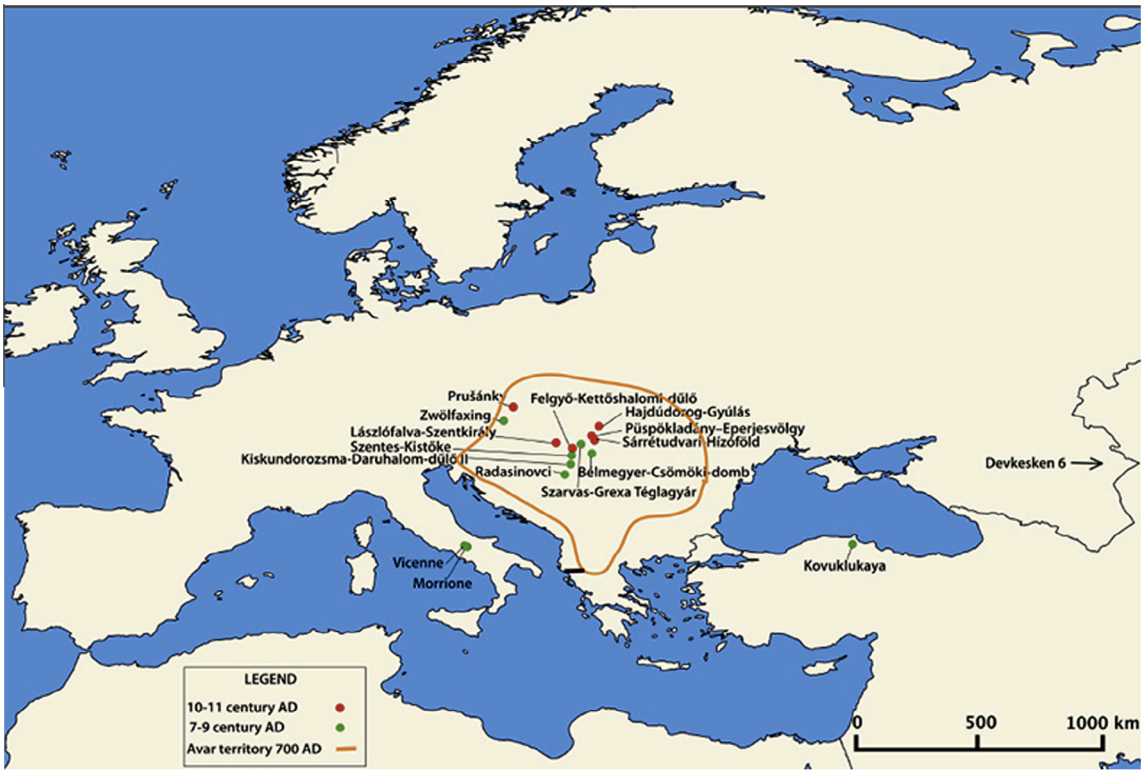

Fig. 1. Location of archaeological sites listed in Table 1.

tical to the leprosy found in Byzantine Anatolia (Table 1) and the main sub-genotype found today in Turkey and the near East (Monot et al., 2009). Two Hungarian samples from the 9th and 10th centuries were of type 3M. Recent whole genome sequencing indicates that 3K strains may form a separate branch of the *M. leprae* phylogenetic tree (Schuenemann et al., 2013), termed branch 0. However, that study was primarily based on strains from Northern and Western Europe, and included only two 3K strains that were from modern China and New Caledonia. Therefore, further work is needed to clarify the phylogeny of the type 3 sub-genotypes. Overall, our biomolecular diagnosis of early cases of leprosy from Hungary, Austria, the Czech Republic, Italy, Croatia, and Turkey suggests that the migration of the Avars from Central Asia into Byzantium and Central Europe was associated with a further wave of leprosy in local populations not previously exposed to the disease.

The few sub-genotypes obtained from Northern and Western Europe are 3I or 2F (Economou et al., 2013; Schuenemann et al., 2013; Taylor et al., 2013; Mendum et al., 2014). These genotypes are from at least two lineages that were associated with the Mediaeval rise in leprosy described in North West Europe and appear to be associated with Nordic and Saxon populations. However, more work is needed to confirm this. The 3I lineage is of special interest as it has been reported in a later medieval burial (Taylor et al., 2009) and is still found in the southern states of the USA (Truman et al., 2011), whence it was probably carried by early European settlers.

The subsequent decline of leprosy in Western Europe cannot readily be explained. Recent analyses of whole genomes retrieved from several European archaeological sites and their comparison with modern isolates (Schuenemann et al., 2013) found no obvious mutations in genes related to virulence or pathogenesis, and no additional pseudogenes in the ancient genomes. Considering host susceptibility, people with leprosy have an impaired immune status, and thus may have been more prone to other infections. This could have included epidemics such as the European 14th century outbreak of the Black Death, caused by *Yersinia pestis* and believed to have killed between one and two-thirds of the European population, including two million people in England. It is likely that the Black Death adversely afflicted the social support networks of *leprosaria*, such as the clergy, individual patrons and physicians. Under such conditions, the suggestion has arisen that a subsequent improvement in socio-economic conditions, coupled with the innate resistance of the surviving population, resulted in a shift to the tuberculoid end of the disease spectrum in those exposed to leprosy (Manchester, 1984). Additionally, it is probable that the increased urbanisation and population density that occurred from the late 15th century resulted in tuberculosis killing leprosy patients (Donoghue et al., 2005a). Either of these two factors could break the transmission of infection. Epidemiological analysis shows that both theories are feasible (Hohmann and Voss-Böhme, 2013).

In conclusion, the genotyping data currently available support the suggestion that different *M. leprae* strains from Central Asia or Asia Minor were introduced into Europe during the early medieval period, associated with the westward migration of Avars. However, more evidence is needed to determine whether microbial virulence or host factors were responsible for the subsequent large rise in the incidence of leprosy in Europe and its subsequent decline.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2015.02.001.

References

Arcini, C., Artelius, T., 1993. Äldsta fallet av spetälska i Norden. Arkeologi i Sverige 2, 55–71.

Bakija-Konsuo, A., Mulić, R., 2011. The history of leprosy in Dubrovnik: an overview. Int. J. Dermatol. 50, 1428–1431. http://dx.doi.org/10.1111/j.1365-4632.2011.05018.x.

Belcastro, M.G., Mariotti, V., Facchini, F., Dutour, O., 2005. Leprosy in a skeleton from the 7th century necropolis of Vicenne-Campochiaro (Molise, Italy). Int. J. Osteoarchaeol. 15, 431–448. http://dx.doi.org/10.1002/oa.799.

Blau, S., Yagodin, V., 2005a. Osteoarchaeological evidence for leprosy from Western Central Asia. Am. J. Phys. Anthropol. 126, 150–158. http://dx.doi.org/10.1002/ajpa.20121.

Blau, S., Yagodin, V., 2005b. AMS radiocarbon dates of kurgans located on the Ust'-Yurt plateau, Uzbekistan. Radiocarbon 47, 235–241.

Blondiaux, J., Dürr, J., Khouchaf, L., Eisenberg, L.E., 2002. In: Roberts, C.A., Lewes, M.E., Manchester, K. (Eds.), The Past and Present of Leprosy. Archaeological, Historical, Palaeopathological and Clinical Approaches. BAR International Series 1054, Archaeopress, Oxford, UK, pp. 105–110.

Brothwell, D.R., Powers, R., Hirst, S.M., Eisenberg, L.E., 2000. Cannington Cemetery: Excavations 1962–3 of prehistoric, Roman, Post-Roman and Later Features at Cannington Park Quarry, Near Bridgewater, Somerset, Britannia Monograph Series No. 17. Society for the Promotion of Roman Studies, London, pp. 195–256.

Buzhilova, A., 2002. In: Roberts, C.A., Lewes, M.E., Manchester, K. (Eds.), The Past and Present of Leprosy. Archaeological, Historical, Palaeopathological and Clinical Approaches, BAR International Series 1054. Archaeopress, Oxford, pp. 123–133.

Csóri, Z., Donoghue, H.D., Marcsik, A., 2009. Leprosy in the 10th–13th century AD in Eastern Hungary. Annuaire Roumain d'Anthropologie 46, 3–11.

Dols, M.W., 1979. Leprosy in Medieval Arabic medicine. J. Hist. Med. Allied Sci. 34, 314–333. http://dx.doi.org/10.1093/jhmas/XXXIV.3.314.

Donoghue, H.D., Holton, J., Spigelman, M., 2001. PCR primers that can detect low levels of *Mycobacterium leprae* DNA. J. Med. Microbiol. 50, 177–182.

Donoghue, H.D., Gladykowska-Rzeczycka, J., Marcsik, A., Holton, J., Spigelman, M., 2002. In: Roberts, C.A., Lewes, M.E., Manchester, K (Eds.), The Past and Present of Leprosy. Archaeological, Historical, Palaeopathological and Clinical Approaches, BAR International Series 1054. Archaeopress, Oxford, pp. 271–285.

Donoghue, H.D., Marcsik, A., Matheson, C., Vernon, K., Nuorala, E., Molto, J.E., Greenblatt, C.L., Spigelman, M., 2005a. Co-infection of *Mycobacterium tuberculosis* and *Mycobacterium leprae* in human archaeological samples: a possible explanation for the historical decline of leprosy. Proc. R. Soc. B 272, 389–394. http://dx.doi.org/10.1098/rspb.2004.296.

Donoghue, H.D., Marcsik, A., Molnár, E, Paluch, T., Szalontai, Cs., 2005b. "Lepra nyomai a kiskundorozsmai avar temetőből. Előzetes beszámoló. Hadak útján" – Népességek és iparok a népvándorlás korában (Osteological signs of leprosy from the Avar period series of Kiskunorozsma. Preliminary report. In: In the Way of the Armies–Population and Industries in the Migration Period), pp. 171–186.

Economou, C., Kjellström, A., Lidén, K., Panagopoulos, I., 2013. Ancient DNA reveals an Asian type of *Mycobacterium leprae* in Medieval Scandinavia. J. Archaeol. Sci. 40, 465–470. http://dx.doi.org/10.1016/j.jas.2012.07.005 (Corrigendum J. Archaeol. Sci., 40, 2867).

Elliot Smith, G., Dawson, W.R., 1924. Egyptian Mummies. George Allen & Unwin Ltd., London.

Faget, G.H., Mayoral, A., 1944. Bone changes in leprosy: a clinical and roentgenologic study of 505 cases. Radiology 42, 1–13.

Haas, C.J., Zink, A., Pálfi, G., Szeimies, U., Nerlich, A.G., 2000. Detection of leprosy in ancient human skeletal remains by molecular identification of *Mycobacterium leprae*. Am. J. Clin. Pathol. 114, 428–436.

Hohmann, H., Voss-Böhme, A., 2013. The epidemiological consequences of leprosy–tuberculosis co-infection. Math. Biosci. 241, 225–237. http://dx.doi.org/10.1016/j.mbs.2012.11.008.

Holló, G., Szathmáry, L., Marcsik, A., Barta, Z., 2008. History of the peoples of the Great Hungarian Plain in the first millennium: a craniometric point of view. Hum. Biol. 80, 655–667. http://dx.doi.org/10.3378/1534-6617-80.6.655.

Kjellström, A., 2010. Possible cases of leprosy and tuberculosis in medieval Sigtuna, Sweden. Int. J. Osteoarchaeol. 22, 261–283. http://dx.doi.org/10.1002/oa.1204.

Lechat, M.F., 1999. The paleopathology of leprosy: an overview. Int. J. Lepr. Other Mycobact. Dis. 67, 460–470.

Lee, O.Y.-C., Bull, I.D., Molnár, E., Marcsik, A., Pálfi, G., Donoghue, H.D., Besra, G.S., Minnikin, D.E., 2012. Integrated strategies for the use of lipid biomarkers in the diagnosis of ancient mycobacterial disease. In: Mitchell, P.D., Buckberry, J. (Eds.), Proceedings of the Twelfth Annual Conference of the British Association for Biological Anthropology and Osteoarchaeology, Department of Archaeology and Anthropology University of Cambridge 2010, BAR International Series 2380. Archaeopress, Oxford, UK, pp. 63–69, ISBN 978-1-4073-0970-5.

Manchester, K., 1984. Tuberculosis and leprosy in antiquity: an interpretation. Med. Hist. 28, 162–173. http://dx.doi.org/10.1017/S0025727300035705.

Manchester, K., Roberts, C., 1989. The palaeopathology of leprosy in Britain: a review. World Archaeol. 21, 265–272. http://dx.doi.org/10.1080/00438243.1989.9980105.

Marcsik, A., Molnár, E., Ősz, B., Donoghue, H., Zink, A., Pálfi, G., 2009. Adatok a lepra, tuberculosis és syphilis magyarországi paleopatológiájához (The palaeopathology of leprosy, tuberculosis and syphilis in Hungary). Folia Anthropol. 8, 5–35.

Mariotti, V., Dutour, O., Belcastro, M.G., Facchini, F., Brasili, P., 2005. Probable early presence of leprosy in a Celtic skeleton of the 4th–3rd century BC (Casalecchio di Reno, Bologna, Italy). Int. J. Osteoarchaeol. 15, 311–325. http://dx.doi.org/10.1002/oa.775.

Mark, S., 2002. Alexander the great, seafaring, and the spread of leprosy. J. Hist. Med. Allied Sci. 57, 285–311. http://dx.doi.org/10.1093/jhmas/57.3.285.

Matheson, C.D., Vernon, K.K., Lahti, A., Fratpietro, R., Spigelman, M., Gibson, S., Greenblatt, C.L., Donoghue, H.D., 2010. 2009 molecular exploration of the First-Century Tomb of the Shroud in Akeldama, Jerusalem. PLoS ONE 4, e8319. http://dx.doi.org/10.1371/journal.pone.0008319 (erratum. In: PLoS One 5(4). doi: 10.1371/annotation/32ada7b9-3772-4c08-9135-b5c0933f0b5e. Zissu, Boaz [added]).

McMichael, A.J., 2001. Human culture, ecological change, and infectious disease: are we experiencing history's fourth great transition? Ecosyst. Health 7, 107–115. http://dx.doi.org/10.1046/j.1526-0992.2001.007002107.x.

McMichael, A.J., 2004. 2004 environmental and social influences on emerging infectious diseases: past, present and future. Phil. Trans. R. Soc. Lond. B 359, 1049–1058. http://dx.doi.org/10.1098/rstb.1480.

Mendum, T.A., Schuenemann, V.J., Roffey, S., Taylor, G.M., Wu, H., Singh, P., Tucker, K., Hinds, J., Cole, S.T., Kierzek, A.M., et al., 2014. 2014 *Mycobacterium leprae* genomes from a British medieval leprosy hospital: towards understanding an ancient epidemic. BMC Genomics 15, 270 (http://www.biomedcentral.com/1471-2164/15/270).

Minnikin, D.E., Besra, G.S., Lee, O.-Y.C., Spigelman, M., Donoghue, H.D., 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., Piombini-Mascali, D. (Eds.), Yearbook of Mummy Studies, vol. 1. Verlag Dr. Friedrich Pfeil, Münich, Germany, pp. 109–114.

Møller-Christensen, V., 1961. Bone Changes in Leprosy. Munksgaard, Copenhagen.

Molto, J.E., 2002. In: Roberts, C.A., Lewes, M.E., Manchester, K. (Eds.), The Past and Present of Leprosy. Archaeological, Historical, Palaeopathological and Clinical Approaches, BAR International Series 1054. Archaeopress, Oxford, pp. 179–192.

Monot, M., Honoré, N., Garnier, T., Araoz, R., Coppée, J.-Y., Lacroix, C., Sow, S., Spencer, J.S., Truman, R.W., Williams, D., et al., 2005. On the origin of leprosy. Science 308, 1040–1042. http://dx.doi.org/10.1126/science/1109759.

Monot, M., Honoré, N., Garnier, T., Zidane, N., Sherafi, D., Paniz-Mondolfi, A., Matsuoka, M., Taylor, G.M., Donoghue, H.D., Bouwman, A., et al., 2009. Comparative genomic and phylogeographic analysis of *Mycobacterium leprae*. Nat. Genet. 41, 1282–1289. http://dx.doi.org/10.1038/ng.477.

Ortner, D.J., 2003. Identification of Pathological Conditions in Human Skeletal Remains, second ed. Academic Press, Amsterdam.

Ortner, D.J., Putschar, W.J.G., 1985. Identification of Pathological Conditions in Human Skeletal Remains. Smithsonian Institution Press, Washington.

Pálfi, G., 1991. The first osteoarchaeological evidence of leprosy in Hungary. Int. J. Osteoarchaeol. 1, 99–102. http://dx.doi.org/10.1002/oa.1390010205.

Pálfi, G., Molnár, E., 2009. The paleopathology of specific infectious diseases from Southeastern Hungary: a brief overview. Acta Biol. Szeged 53, 111–116 (http://www.sci.u-szeged.hu/ABS).

Pinhasi, R., Foley, R., Donoghue, H.D., 2006. Reconsidering the antiquity of leprosy. Science 312, 846. http://dx.doi.org/10.1126/science.312.5775.846a.

Rafi, A., Spigelman, M., Stanford, J., Lemma, E., Donoghue, H., Zias, J., 1994. DNA of *Mycobacterium leprae* detected by PCR in ancient bone. Int. J. Osteoarchaeol. 4, 287–290. http://dx.doi.org/10.1002/oa.1390040403.

Reader, R., 1974. New evidence for the antiquity of leprosy in early Britain. J. Archaeol. Sci. 1, 205–207. http://dx.doi.org/10.1016/0305-4403(74)90043-0.

Redman, J.E., Shaw, M.J., Mallet, A.I., Santos, A.L., Roberts, C.A., Gernaey, A.M., Minnikin, D.E., 2009. Mycocerosic acid biomarkers for the diagnosis of tuberculosis in the Coimbra skeletal collection. Tuberculosis 89, 267–277. http://dx.doi.org/10.1016/j.tube.2009.04.001.

Robbins Schug, G., Blevins, K.E., Cox, B., Gray, K., Mushrif-Tripathy, V., 2013. Infection, disease, and biosocial processes at the end of the Indus civilization. PLoS ONE 8, e84814. http://dx.doi.org/10.1371/journal.pone.0084814.

Roberts, C.A., 2002. The antiquity of leprosy in Britain: the skeletal evidence. In: Roberts, C.A., Lewes, M.E., Manchester, K. (Eds.), The Past and Present of Leprosy. Archaeological, Historical, Palaeopathological and Clinical Approaches, BAR International Series 1054. Archaeopress, Oxford, pp. 213–221.

Rubini, M., Zaio, P., 2009. Lepromatous leprosy in an early medieval cemetery in Central Italy (Morrione, Campochiaro, Molise, 6th–8th century AD. J. Archaeol. Sci. 36, 2771–2779. http://dx.doi.org/10.1016/j.jas.2009.09.002.

Rubini, M., Zaio, P., 2011. Warriers from the East. Skeletal evidence of warfare from a Lombard-Avar cemetery in central Italy (Campochiaro, Molise, 6th–8th century AD). J. Archaeol. Sci. 38, 1551–1559. http://dx.doi.org/10.1016/j.jas.2011.02.020.

Rubini, M., Erdal, Y.S., Spigelman, M., Zaio, P., Donoghue, H.D., 2012. Paleopathological and molecular study on two cases of ancient childhood leprosy from the Roman and Byzantine empires. J. Osteoarchaeol. Int. http://dx.doi.org/10.1002/oa.2242.

Rubini, M., Zaio, P., Roberts, C., 2014. Tuberculosis and leprosy in Italy. New skeletal evidence. HOMO – J Compar. Hum. Biol. 65, 13–32. http://dx.doi.org/10.1016/j.jchb.2013.07.006.

Schuenemann, V.J., Singh, P., Mendum, T.A., Krause-Kyora, B., Jäger, G., Bos, K.I., Herbig, A., Economou, C., Benjak, A., Busso, P., et al., 2013. Genome-wide comparison of Medieval and modern *Mycobacterium leprae*. Science 341, 179–183. http://dx.doi.org/10.1126/science.1238286.

Skinsnes, O.K., Chang, P.H., 1985. Understanding of leprosy in ancient China. Int. J. Lepr. Other Mycobact. Dis. 53, 289–307.

Taylor, G.M., Donoghue, H.D., 2011. Multiple loci variable number tandem repeat (VNTR) analysis (MLVA) of *Mycobacterium leprae* isolates amplified from European archaeological human remains with lepromatous leprosy. Microb. Infect. 13, 923–929. http://dx.doi.org/10.1016/j.micinf.2011.05.003.

Taylor, G.M., Watson, C.L., Bouwman, A.S., Lockwood, D.N.J., Mays, S.A., 2006. Variable nucleotide tandem repeat (VNTR) typing of two palaeopathological cases of lepromatous leprosy from mediaeval England. J. Archaeol. Sci. 33, 1569–1579. http://dx.doi.org/10.1016/j.jas.2006.02.008.

Taylor, G.M., Blau, S., Mays, S., Monot, M., Lee, O.Y.-C., Minnikin, D.E., Besra, G.S., Cole, S.T., Rutland, P., 2009. *Mycobacterium leprae* genotype amplified from an archaeological case of lepromatous leprosy in Central Asia. J. Archaeol. Sci. 36, 2408–2414. http://dx.doi.org/10.1016/j.jas.2009.06.026.

Taylor, G.M., Tucker, K., Butler, R., Pike, A.W.G., Lewis, J., Roffey, P.M., Lee, O.Y.-C., Wu, H.H.T., Minnikin, D.E., Besra, G.S., et al., 2013. Detection and strain typing of ancient *Mycobacterium leprae* from a Medieval leprosy hospital. PLoS ONE 8, e62406. http://dx.doi.org/10.1371/journal.pone.0062406.

Truman, R.W., Singh, P., Sharma, R., Busso, P., Rougemont, J., Paniz-Mondolfi, A., Kapopoulou, A., Brisse, S., Scollard, D.M., Gillis, T.P., Cole, S.T., 2011. Probable zoonotic leprosy in the Southern United States. N. Engl. J. Med. 364, 1626–1633. http://dx.doi.org/10.1056/NEJMoa1010536.

Watson, C.L., Popescu, E., Boldsen, J., Slaus, M., Lockwood, D.N.J., 2009. Single nucleotide polymorphism analysis of European archaeological *M. leprae* DNA. PLoS ONE 4 (10), e7547. http://dx.doi.org/10.1371/journal.pone.0007547 (authors corrected: 10.1371/annotation/1b400b6e-8883-436c-b3c4-00e1ec2db101).

Weng, X., Xing, Y., Liu, J., Wang, Y., Ning, Y., Ming, L., Wu, W., Zhang, L., Li, W., Vander Heiden, J., et al., 2013. Molecular, ethno-spatial epidemiology of leprosy in China: novel insights for tracing leprosy in endemic and non endemic provinces. Infect. Genet. Evol. 14, 361–368. http://dx.doi.org/10.1016/j.meegid.2012.12.009.

Zhang, G., Rong, X., 1998. A concise history of the Turfan Oasis and its exploration. Asia Major (Third Series) 11, 13–36.

Zias, J., 1985. Leprosy in the Byzantine monasteries of the Judean Desert. Korot 9, 242–248.