Chapter 2

The Distribution and Origins of Ancient Leprosy

Helen D. Donoghue, G. Michael Taylor,
Tom A. Mendum, Graham R. Stewart, Leen Rigouts,
Oona Y-C. Lee, Houdini H.T. Wu,
Gurdyal S. Besra and David E. Minnikin

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75260

Abstract

Human leprosy is primarily caused by *Mycobacterium leprae*, but also by the related '*M. lepromatosis*'. Ancient leprosy can be recognised in archaeological materials by the paleopathology associated with multi-bacillary or lepromatous forms of the disease. Whole *M. leprae* genomes have been obtained from human skeletons, and diagnostic aDNA fragments have been recovered. The derived *M. leprae* phylogenies, based on single nucleotide polymorphisms, mirror past human migrations, as *M. leprae* is usually an obligate pathogen. The detection of *M. leprae* in historical leprosy cases is assisted by the hydrophobic *M. leprae* cell envelope, which is composed of unusual lipids that can be used as specific biomarkers. Lipid biomarkers are more stable than aDNA and can be detected directly without amplification. Indigenous human leprosy is extinct in Western Europe, but recently, both *M. leprae* and '*M. lepromatosis*' were found in British red squirrels. Leprosy may also be found in nine-banded armadillos (*Dasypus novemcinctus*) where it can cause a zoonotic human infection. Certain leprosy-like diseases, caused by uncultivable species in cats, for example, may be related to *M. leprae*. The closest extant relatives of leprosy bacilli are probably members of the *M. haemophilum* taxon, emerging pathogens with genomic and lipid biomarker similarities.

Keywords: ancient DNA, lipid biomarkers, genotyping, leprosy, paleopathology, evolution

1. Introduction

Leprosy (Hansen's disease) is a chronic infectious disease that has been recognised over millennia. In the majority of human cases, it is caused by *Mycobacterium leprae*, but recently a

related organism, '*M. lepromatosis*', has also been implicated [1] and appears to cause diffuse lepromatous leprosy (DLL). Both organisms are obligate pathogens that are uncultivable in cell-free growth media. Although '*M. lepromatosis*' has been the subject of many recent publications [2–5], there is still discussion about whether it is a distinct species [6]; currently, it is a name without standing in nomenclature (http://www.bacterio.net/-nonvalid.html). Leprosy is primarily a disease of peripheral nerves and skin, but it also affects bones. The genomes of *M. leprae* and '*M. lepromatosis*' have been sequenced, and it is clear that they diverged from a common ancestor many millennia ago [7, 8]. The genome of '*M. lepromatosis*' confirms a close but distinct relationship with *M. leprae*, and both organisms can also cause disease in animals, such as armadillos and squirrels [9–12]. The closest ancestors of these leprosy bacilli are probably relatives of *M. haemophilum* that has genomic and lipid biomarker similarities [13–16].

Initially, ancient leprosy was recognised by the paleopathology associated with multi-bacillary or lepromatous forms of the disease [17, 18]. Leprosy causes skeletal changes in the rhinomaxillary area, including pitting and perforation in the palate, resorption of the nasal spine and the maxilla leading to loss of the upper teeth. The tubular bones of the hands and feet are frequently involved. In the tibia and fibula, inflammatory periostitis can be recognised; the metatarsals and metacarpals are often resorbed so these small bones develop a pencil shape. In sub-adult individuals afflicted with multibacillary leprosy, the development of the secondary dentition can be affected, leading to a rare condition, *leprogenic odontodysplasia* (LO), where the incisor teeth exhibit a characteristic root constriction [19]. Intriguingly, this has been seen only in archaeological cases and not in a clinical setting. Cases have been described from medieval Denmark [20] and in four individual medieval inhumations from the St. Mary Magdalen, Winchester leprosarium [21]. Subtle skeletal changes like grooving on the volar surfaces of the proximal phalanges may also accompany paucibacillary forms of leprosy that cause digital contracture or loss of pain sensation [22].

Suspected leprosy cases can be confirmed by the detection of *M. leprae* ancient DNA (aDNA) [23, 24] and further characterised by repetitive DNA sequences and genotyping [25, 26]. The aDNA detection of *M. leprae* in historical cases is probably assisted by the protective presence of unusual lipids in the *M. leprae* cell envelope. These lipids can be used as specific biomarkers; they are more stable than aDNA and can be directly detected without amplification (*vide infra*). Lipid biomarkers have been used to confirm aDNA findings [21, 27–29]. However, due to their stability, lipid biomarkers can also confirm a diagnosis of leprosy initially based on paleopathology, even in the absence of aDNA [30].

2. Causes and distribution of modern leprosy

2.1. *Mycobacterium leprae*

M. leprae, the main cause of leprosy in humans, is a slow-growing intracellular *Mycobacterium* and the average incubation period of the disease is about 5 years, although symptoms may occur within 1 year or up to 20 years after infection [31]. Leprosy mainly affects the skin, peripheral nerves, the mucosa of the upper respiratory tract and the eyes, as *M. leprae* has a tropism for

Schwann cells in nerves and macrophages in the skin [32]. The infection is transmitted by direct contact with untreated cases or healthy carriers or via infectious aerosols [33]. The clinical presentation of leprosy depends upon the cell-mediated immune (CMI) response to infection. If the host has an effective CMI response, few lesions develop, and there are only scanty bacilli in the tissues. However, some patients are anergic to *M. leprae*, so develop lepromatous leprosy with ineffective antibodies, a high bacterial load and multiple lesions. The clinical presentation of leprosy in a patient can vary over time, so there are borderline leprosy types where the immune response is unstable. It can show a wide range of clinical presentations from tuberculoid leprosy (TT) through borderline forms: borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL) to lepromatous leprosy (LL) [34]. A recent World Health Organization classification scheme recognises a simplified two-category system of either paucibacillary or multibacillary forms of leprosy [35]. The histopathology of skin lesions varies from compact granulomas to diffuse infiltration of dermis, which largely depend upon the immune status of the patient and may not be in agreement with the clinical diagnosis [36, 37]. The mycobacterial antigens can activate a chronic inflammatory response that is exacerbated by pro-inflammatory cytokines. Therefore, in late stages of leprosy, there may be no *M. leprae* bacilli in the tissues, but residual mycobacterial antigens can drive an inflammatory response that causes neurological damage [38].

2.2. '*Mycobacterium lepromatosis*'

'*M. lepromatosis*' appears to have a tropism for endothelial cells and can give rise to vasculitis and necrotic erythema. It seems to be less common than *M. leprae* and was initially believed to be geographically restricted to patients from Mexico and the Caribbean, where it was identified in patients suffering from diffuse lepromatous leprosy (DLL) [1, 39–41]. It was subsequently recognised in Brazil, Myanmar, Canada and Singapore and in mixed infections with *M. leprae* [3, 4]. Symptoms, characteristic of 'Lucio's phenomenon', have been associated with '*M. lepromatosis*' [1, 40, 42]. A case of two Mexican siblings infected with '*M. lepromatosis*' indicates facile transmission [5, 6]. However, '*M. lepromatosis*' has recently been found in the wild Eurasian red squirrel, *Sciurus vulgaris*, in the British Isles, from England, Scotland and Wales [11, 12]. In addition, *M. leprae* was found in red squirrels on the Isle of Wight and Brownsea Island, close to the south coast of England [43, 44]. This was very surprising, as although indigenous leprosy was prevalent in the human population of the British Isles in the first millennium (CE), it is now believed to be extinct. In these modern squirrels, the macroscopic signs and histopathology were characteristic of lepromatous leprosy, but no pathological differences were noted between infections caused by '*M. lepromatosis*' or *M. leprae* [12, 45]. The strain of '*M. lepromatosis*' in British wild squirrels is genetically distinguishable from Mexican strains found in modern day humans, and it appears that these strains diverged from a common ancestor about 26,000 years ago [12]. However, the *M. leprae* strain found in British red squirrels is similar to a strain found in human remains from a mediaeval leprosy hospital in Winchester [46], only 70 km from the Isle of Wight and Brownsea Island. One suggestion is that, in the past, humans may have been infected through direct contact with red squirrels as these were prized for their meat and fur [12]. They were also kept as pets, as is evident from various illustrated medieval manuscripts and art, for example 'A Lady with a Squirrel and a Starling' by Holbein the Younger (painted ca. 1526–1528, National Portrait Gallery, London).

2.3. Nature and distribution of *M. leprae* genotypes

Major collaborative studies based on single nucleotide polymorphism (SNP) typing have established that modern *M. leprae* consists of four distinct genotypes that are associated with different human populations [47]. It is believed that the ancestral precursor of *M. leprae* experienced an evolutionary bottleneck and thereafter developed independently in different human populations [26, 48]. In Europe, indigenous leprosy is now largely extinct, so a further study also looked at *M. leprae* from archaeological cases using aDNA methods [26]. This identified SNP type 3 cases from various European countries for the first time, including Denmark, Hungary, Croatia, Turkey and Britain. Some cases provided subtypes I, M or K. Genotype 3 strains were also found from Roman Egypt and by others in medieval Central Europe [30, 49]. Later studies also reported SNP type 2 strains for the first time in medieval cases from Winchester, UK [21] and from Sweden [50, 51]. Archaeological remains from Japan yielded a SNP type 1 from that country [52]. Several of the robust cases were subsequently amplified by whole genome sequencing (WGS) [46, 53].

Monot et al. [26] also recognised sub-genotypes from extant cases, thereby enabling more precise associations between *M. leprae*, geographical location and present human populations ranging from China [54] to South America [55]. In a detailed study of modern *M. leprae* that included SNP typing, variable-number-tandem-repeat (VNTR) analysis and WGS, Truman et al. [9] examined 50 patients with leprosy and 33 wild armadillos (*Dasypus novemcinctus*) in the United States, together with reference strains from other parts of the world. Seven *M. leprae* SNP types were detected. The SNP type for some patients with possible exposure by foreign residence was typical of *M. leprae* from foreign locations. The most abundant SNP type was 3I that is generally associated with historical northwest European or American populations. The SNP sub-type 3I-1 strains, with one copy of an 11-bp indel (indel_17915) had ancestral bases, but all other *M. leprae* strains have two copies. Type 3I-2 strains, a development of the ancestral 3I-1 strains, similarly have only one copy of indel ML_17915 and can be identified by base C at position 1527056 instead of base G present in type 3I-1 isolates [9]. These 3I-2 strains were found in all armadillos and most of the indigenous patients so the authors concluded that armadillos act as a reservoir for *M. leprae* and that there is zoonotic spread of leprosy in the Southern United States. As the disease was not present in the New World before European contact, it is assumed that the spread of the disease was linked to human migrations and that armadillos acquired leprosy from human cases [45, 56].

2.4. Transmission of leprosy

Recently it was realised that the enhanced hydrophobicity of tubercle bacilli is a key factor in aerosol transmission [57, 58]. Since it is becoming established that aerosol transmission is a prime mode for the spread of leprosy bacilli [33, 59], the transmissibility of the different manifestations of *M. leprae* should be considered. In a detailed study [33], it was demonstrated that MB/LL cases provided more transmissible bacilli than PB/TT patients. It would be of great interest to compare the relative cell envelope surface lipid composition of LL and TT leprosy bacilli to explore the possibility that the hydrophobicity of LL forms is enhanced or otherwise. It may also be possible to determine directly the relative hydrophobicity of *M. leprae* in biopsy

material, using micro fluorescence methods [60]. The evasion of airways epithelial clearance [33, 59] may be encouraged by enhanced hydrophobicity of infective agents.

3. Recognition, diagnosis and spread of ancient leprosy

3.1. Pathology and recognition of ancient leprosy

Leprosy is primarily a disease of the peripheral nervous system. In the past, the disease would run its natural course, resulting in both specific and nonspecific bony changes plus paleopathology due to secondary infections following nerve damage [17, 18, 61]. Ancient leprosy is typically recognised by the presentation known as *facies leprosa* or rhinomaxillary syndrome, in which the nasopharynx is remodelled, the nasal spine and palate are resorbed, and eventually also the maxilla, leading to loss of the upper teeth. There are changes to the tubular bones of the hands and feet including osteoporosis caused by disuse, pitting and perforation. The long bones of the lower leg also show paleopathology associated with inflammatory periostitis [30, 62–64].

M. leprae ancient DNA (aDNA) was first detected in skeletal remains with typical leprosy paleopathology soon after the introduction of PCR [23]. Subsequently, many further paleopathological cases of leprosy were confirmed by *M. leprae* aDNA from across Europe and the Middle East [24–27, 30, 49–51, 64–69]. Specific *M. leprae* short DNA sequences were targeted as ancient DNA (aDNA) becomes highly fragmented over time [70]. *M. leprae* aDNA amplification has confirmed leprosy and enabled genotyping of isolates from Europe, Byzantine Turkey and Roman Egypt (**Table 1**). As additional methodologies were developed, different *M. leprae* strains were distinguished by microsatellite analysis based on aDNA repetitive sequences [27, 71] and now whole *M. leprae* genomes have been obtained from historical human skeletons [46, 53]. The results of aDNA amplification studies, WGS and lipid biomarker detection are summarised in **Table 1**.

3.2. The potential of lipid biomarkers

The detection of *M. leprae* in historical leprosy cases is assisted by the *M. leprae* cell envelope, which is composed of unusual lipids some of which can be used as specific biomarkers (**Figures 1–3**). The mycolic acids of *M. leprae* are restricted to homologous α- and ketomycolates [79, 80], whose major components are shown in **Figure 1**.

Characteristic mycocerosic acids are components of both phthiocerol dimycocerosate waxes (PDIMs) (**Figure 2**) [81–83] and so-called phenolic glycolipids (PGLs) (**Figure 3**) [82–85]. *M. leprae* mycocerosates unusually include major amounts of a C_{34} component, accompanied by small proportions of a C_{33} acid (**Figure 2**). *M. haemophilum* produces a PGL with the same two internal sugars (3-*O*-Me-rhamnose and 2,3-di-*O*-Me-rhamnose), but in reversed order and with different linkages (**Figure 3**). Besra et al. [13] concluded that this mycocerosate profile was essentially the same, thereby revealing a close phylogenetic link between *M. leprae* and *M. haemophilum* for the first time.

Century (CE); location: cases	*M. leprae*		*M. leprae* genotype	Notes	Publications
	DNA	Lipids			
1st; Israel, Akeldema, Himmon valley: SC1	+				Matheson et al. [69]
1st–4th; Uzbekistan Devkesken 6: 5b	+	+	3L		Taylor et al. [27]
4th; Egypt, Dakhleh Oasis, Kellis 2: B116 and 7 other samples	+		3K/L/M (B116)		Donoghue et al. [72]; Monot et al. [26]
4th–7th; Israel, Jerusalem: HZ	+				Spigelman and Donoghue [67]
5th–6th; United Kingdom, Great Chesterfield: GC96	+	+	3I-1 (variant)	MTB –	Inskip et al. [73]
6th–7th; Israel, monastery on River Jordan: AR	+				Rafi et al. [23]
6th–8th; Italy, Morrione: T68, T108	1/2 + (T108)				Donoghue et al. [30]
7th; Hungary, Szeged-Kiskundorozsma-Daruhalom dűlő II: KD271, KD517, KD518	3/3+	KD517+	3K (KD271)	KD517 lipids+ and MTB+	Minnikin et al. [29]; Lee et al. [28]; Donoghue et al. [30]
7th; Italy, Vicenne: T18, T31, T144	1/3+ (T18)	2/2+ (T18, T144)		DNA-lipids+ (T144)	Donoghue et al. [30]
7th–8th; Hungary, Szentes-Kistőke: SK11	+				Donoghue et al. [30]
7th–9th; Hungary, Bélmegyer-Csömöki domb: 22	+	+		MTB lipid+	Donoghue et al. [30]; Molnár et al. [74]
7th–9th; Hungary, Szarvas Grexa, Téglagyár: SG-38	+	+			Minnikin et al. [29]; Donoghue et al. [30]
8th–9th; Turkey, Kovuklukaya: 9/1, 11/2, 20/1, 24/1	3/4+ (11/2−)	1/3+ (24/1+)	3K (20/1)		Minnikin et al. [29]; Donoghue et al. [30]
8th–9th; Croatia, Radasinovci: 2A, 3A	+				Watson et al. [49]
8th–9th; Austria, Zwölfaxing: 70, 88	2/2+			MTB DNA+ (88)	Donoghue et al. [30]
9th–10th; Czech Republic, Prušánky: 188	+		3M		Donoghue et al. [30]

Century (CE); location: cases	*M. leprae*		*M. leprae* genotype	Notes	Publications
	DNA	Lipids			
10th; Hungary, Hajdúdorog-Gyúlás: HG-56	+	+			Minnikin et al. [29]; Donoghue et al. [30]
10th; Hungary, Sárrétudvari-Hízóföld: S237	+			Palate+ Toe−	Haas et al. [65]
10th–11th; UK, Norwich: 11287, 11503, 11784	+		3		Watson et al. [49]
10th–11th; Hungary, Püspökladány-Eperjesvölgy 11, 222, 429, 503	+	+ (503)	3K (222) 3M (503)	222 and 503 MTB+	Donoghue et al. [30]
10th–12th; UK, Wharram Percy: G708	+		3		Taylor and Donoghue [71]
11th; Sweden, Björned: A4	+	+		MTB+	Donoghue et al. [72]; Minnikin et al. [29]
10th–12th; Sweden, Sigtuna: 10, 32H, 3077, 3092V, 3093F, 3159Hsin, 3320V, 3401H, F13320, S10V3	7/10+		2F (3092 and 3077) 3I (3093)	WGS	Economou et al. [50, 51]; Schuenemann et al. [46]
11th; Hungary, Felgyő, Kettőshalmi-dűlő: 2467, 3658	1/2+			3658+	Donoghue et al. [30]
11th; Hungary, Lászlófalva-Szentkirály: 79	+			MTB+	Donoghue et al. [30]
11th–12th; UK, Orkney: CC4	+				Taylor et al. [66]
9th–13th; UK, Winchester: Sk2, Sk7, Sk19 Sk8, Sk14, Sk27 Sk18	+	+	3I-1 2F	WGS Sk18 (weak)	Schuenemann et al. [46]; Taylor et al. [21] Roffey *et al*. 2017 [75]
11th–14th; Denmark, Refshale:2, 16, 26, 32, 36	1/5+	+	2F (Refshale16)	Refshale 16+	Schuenemann et al. [46]
12th; Spain, Seville: A43, A120	+				Montiel et al. [76]
12th; Czech Republic, Žatec: AO9611, AO9731	+				Likovsky et al. [77]
12th–14th; Poland, Suraz: A1	+				Donoghue et al. [70]; Witas et al. [78]

Century (CE); location: cases	*M. leprae*		*M. leprae* genotype	Notes	Publications
	DNA	Lipids			
13th–14th; Denmark, Odense: Jorgen 625, 1020	1/2+	+	3I (Jorgen 625)	Jorgen 625+	Schuenemann et al. [46]
13th–16th; UK, Ipswich, Blackfriars: 1914	+		3I* (variant)		Taylor et al. [25, 27]; Taylor and Donoghue [71]
13th–16th; Denmark, Odense: G483	+		3I/J		Watson et al. [49]
15th; Hungary, Szombathely: 10	+	+			Donoghue et al. [72]; Minnikin et al. [29]
15th–18th; Germany, Rain/Lech: R1788, R2208	2/2+				Haas et al. [65]
18th–20th; Japan, Aomori: SK26	+		1		Suzuki et al. [52]

Cases are listed in a chronological order.

Table 1. Detection of ancient leprosy using aDNA and lipid biomarkers.

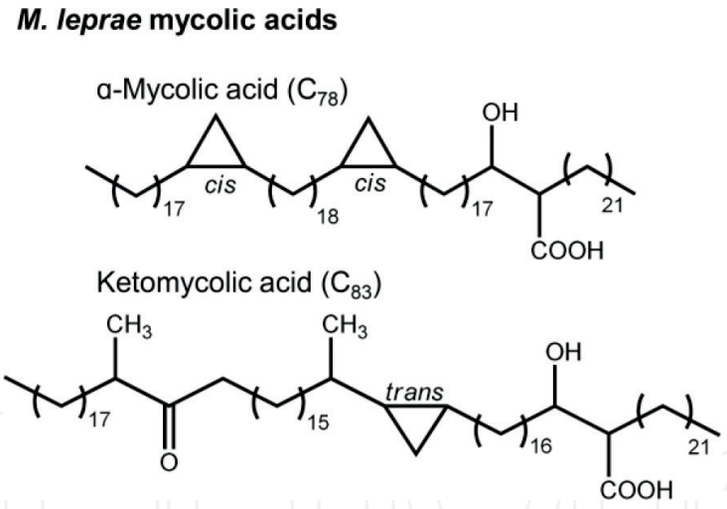

Figure 1. Mycolic acids of *M. leprae*. The main C_{78} α-mycolate and C_{83} ketomycolate are shown; additional homologous components are also present.

The lipid composition of '*M. lepromatosis*' remains to be determined, but limited information is available for *M. haemophilum*. In addition to α- and ketomycolates, *M. haemophilum* appears to have methoxymycolates, on thin-layer chromatography of extracts [86], but in a previous study, the patterns were unclear with material being degraded by acid methanolysis [87]. A gas chromatographic profile of *M. haemophilum* fatty acids [86] displayed an essentially typical mycobacterial profile, including tuberculostearic acid. The analysis was not extended to search for the unusual mycocerosic acids found previously in *M. haemophilum* (**Figure 2**) [13]. The only novel component was an incompletely characterised monounsaturated 2-methyl-branched C_{25} fatty acid and an enhanced proportion of C_{22} docosanoic acid was noted as being

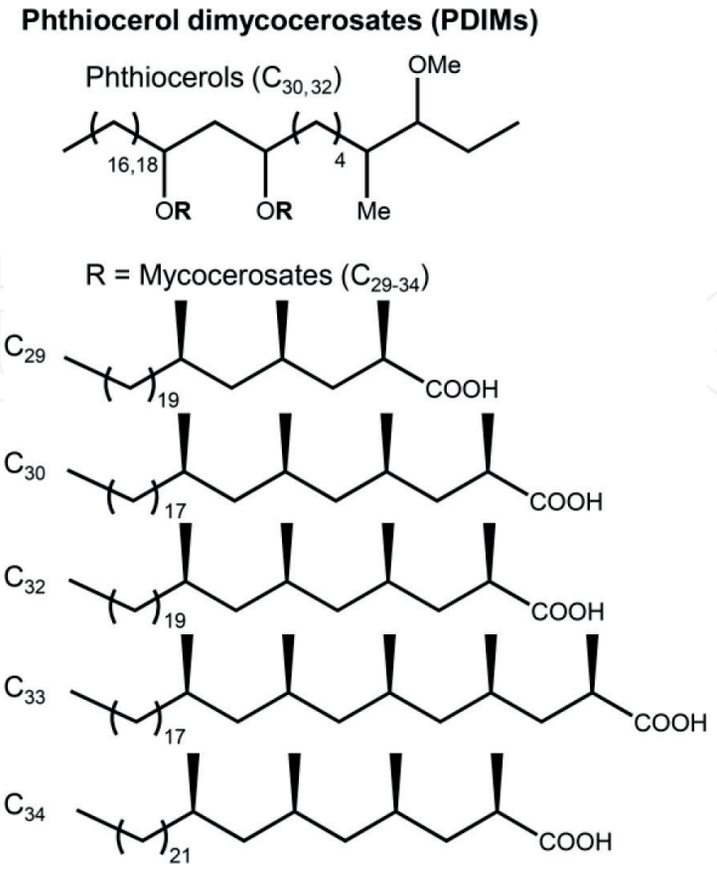

Figure 2. Phthiocerol dimycocerosates (PDIMs) of *M. leprae*. The C_{33} and C_{34} mycocerosates are diagnostic components for *M. leprae* and *M. haemophilum,* but C_{29}, C_{30} and C_{32} acids are shared with members of the *M. tuberculosis* complex [13, 81, 82].

similar to that found in *M. leprae* in a previous study [88]. However, an analysis of three *M. leprae* isolates did not record unusually enhanced proportions of docosanoic acid [80], nor did an additional analysis of *M. haemophilum* fatty acids [89]. It is interesting to compare the profile of uncharacterised fatty acids from *M. haemophilum* in an older study [87] with the more recent study [86]. An unusual large peak, labelled 19A, in the first analysis [87] could possibly correspond to the minor branched C_{25} acid in the later analysis [86]. This unusual C_{25} acid is a potentially valuable biomarker for *M. haemophilum* so its structure and cellular location should be investigated.

The biomarker potential of *M. leprae* lipids has been harnessed by fluorescence high performance liquid chromatography (HPLC) of pyrenebutyric acid (PBA) esters of mycolic acid pentafluorobenzyl (PFB) esters [90] and negative-ion chemical-ionisation gas-chromatography mass-spectrometry (NI-CI GC-MS) of mycocerosate PFB esters [91, 92]. Mycolate HPLC is exemplified in **Figure 4** for standard *M. leprae* and an extract of a skeleton (Sk2) from a mediaeval leprosy hospital near Winchester, UK [21]. Fluorescent mycolate derivatives are recognised by reverse-phase HPLC (**Figure 4A**), collected and analysed by normal phase HPLC to separate the α- and ketomycolate classes (**Figure 4B**). Reverse-phase HPLC provides the size and overall composition of the α-mycolates (**Figure 4C**) and ketomycolates (**Figure 4D**) for comparison with standard *M. leprae.*

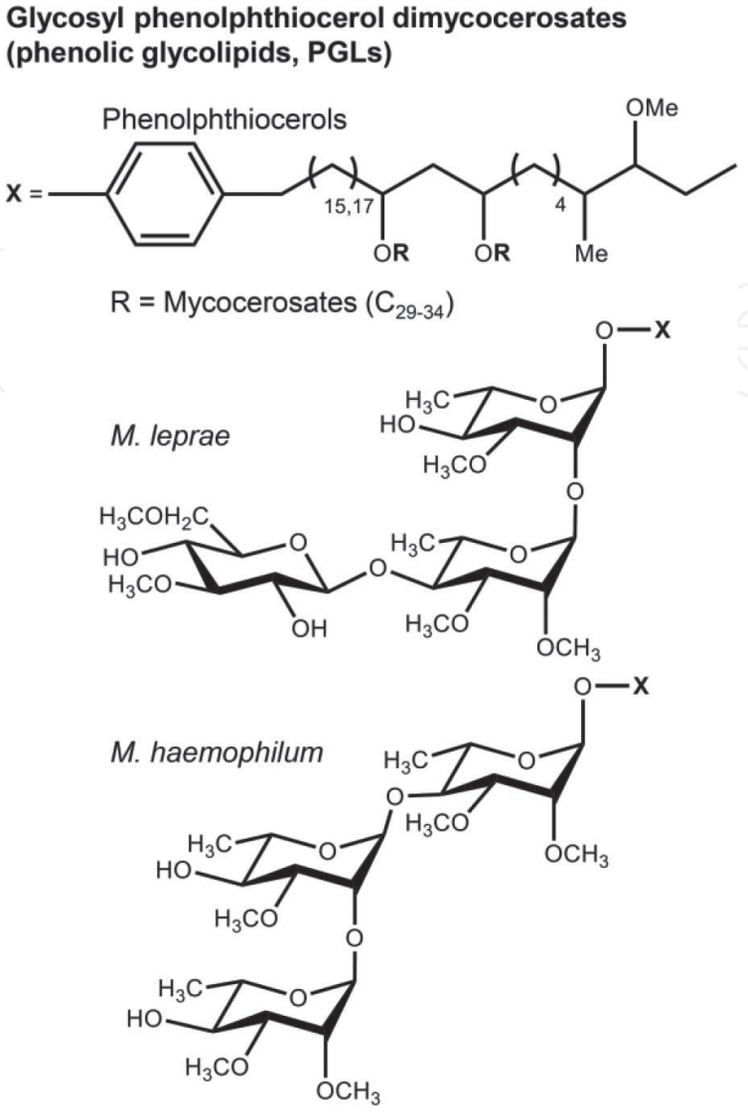

Figure 3. Phenolic glycolipids of *M. leprae* and *M. haemophilum*. The common phenolphthiocerol unit is attached to distinctive trisaccharides that share particular diagnostic sugars, 3-*O*-Me-rhamnose and 2,3-di-*O*-Me-rhamnose [13].

Selected ion monitoring NI-CI GC-MS analyses of mycocerosate PFB esters from Winchester skeleton Sk2 [21] and standard *M. leprae* are shown in **Figure 5**. There is good correspondence between the Sk2 extract and the standard; the Sk2 profile is unpublished work (O.Y-C. Lee, H.H.T. Wu, G.M. Taylor, K. Tucker, R. Butler, S. Roffey, P. Marter, D.E. Minnikin, G.S. Besra, G.R. Stewart, manuscript in preparation). In summary (**Table 1**), aDNA analysis with occasional lipid biomarker support has been successful in characterising ancient leprosy [21, 27, 29].

3.3. Distribution and phylogeny of ancient leprosy

Further, aDNA studies based on *M. leprae* sub-genotypes have given valuable information about the distribution of the disease in different human populations in the past [26]. The earliest known case of leprosy recognised by both skeletal paleopathology and aDNA, was from the early first millennium CE from the Ustyurt Plateau, Uzbekistan [93], with radiocarbon dating that suggests a date between the first and third centuries CE [94]. The *M. leprae*

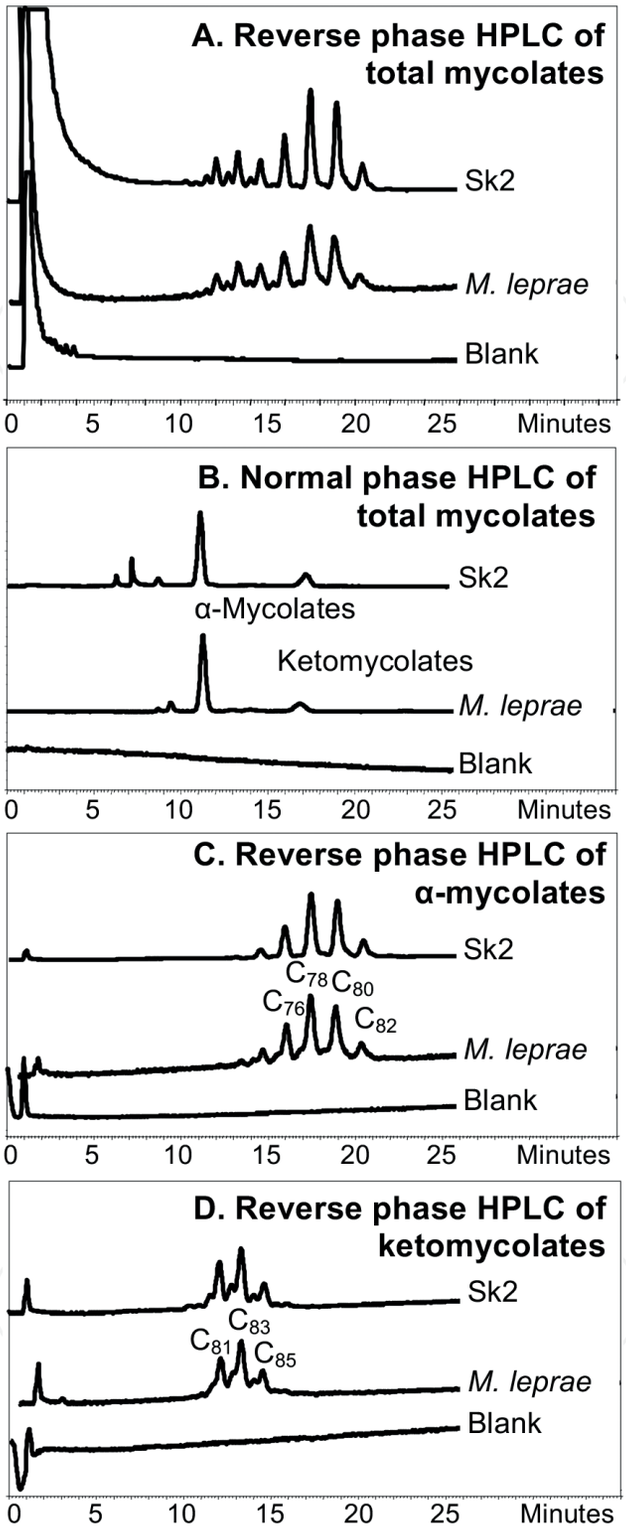

Figure 4. Mycolic acid profiles of Winchester skeleton Sk2. (A) Total mycolates, reverse phase HPLC; (B) collected total mycolates (MAs), normal phase; (C) collected α-mycolates, reverse phase; (D) Collected ketomycolates, reverse phase [21].

aDNA from this location was found to be of sub-genotype 3L [27] and the variable number tandem repeat analysis identified a unique aDNA profile [71]. Sub-genotyping has revealed that in historical Europe, there are clear differences between the leprosy found in human

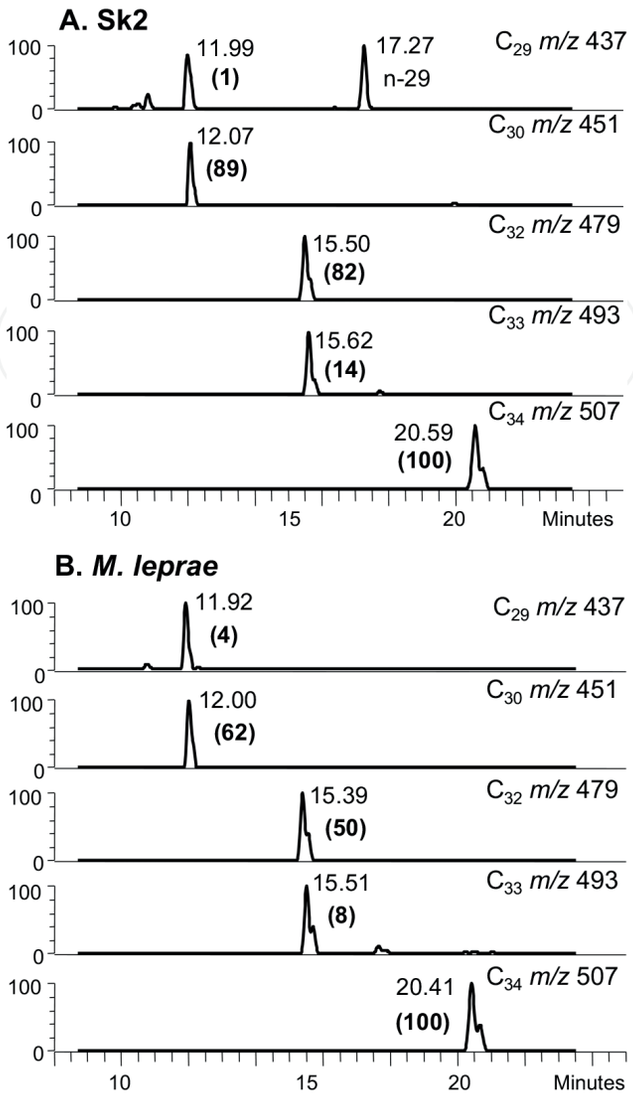

Figure 5. Mycocerosic acid profiles of Winchester skeleton Sk2. Selected ion monitoring NI-CI GC-MS of mycocerosic acid pentafluorobenzyl ester from A, Sk2 and B, *M. leprae* standard.

populations from central and southern Europe, compared with northwest Europe (**Table 1** and **Figure 6**). In Scandinavia and the British Isles, there are examples of *M. leprae* genotypes 2F and 3I [21, 46, 53, 75]. In historical northwest Europe, 3I-1 sub-genotypes were common, but in Hungary, Byzantine Turkey and the Czech Republic, sub-genotypes 3K and 3M were found [30]. It is believed that these differences reflect past human population movements. In northwest Europe, people travelled from Siberia and the Arctic, whereas central Europe was colonised by successive migrations from central Asia *via* ancient routes, such as the so-called Silk Road. WGS of the 3K subtype shows that it belongs to the earliest lineage of extant *M. leprae*, now termed branch 0 [46], and therefore carries characteristics of the most recent common ancestor (MRCA), not found in other groups. The distribution of the various European sub-genotypes is summarised in **Figure 6** and their phylogenetic relationship in **Figure 7**. It would be informative to have more data points for the Mediterranean basin and major countries, such as Spain, France and Germany.

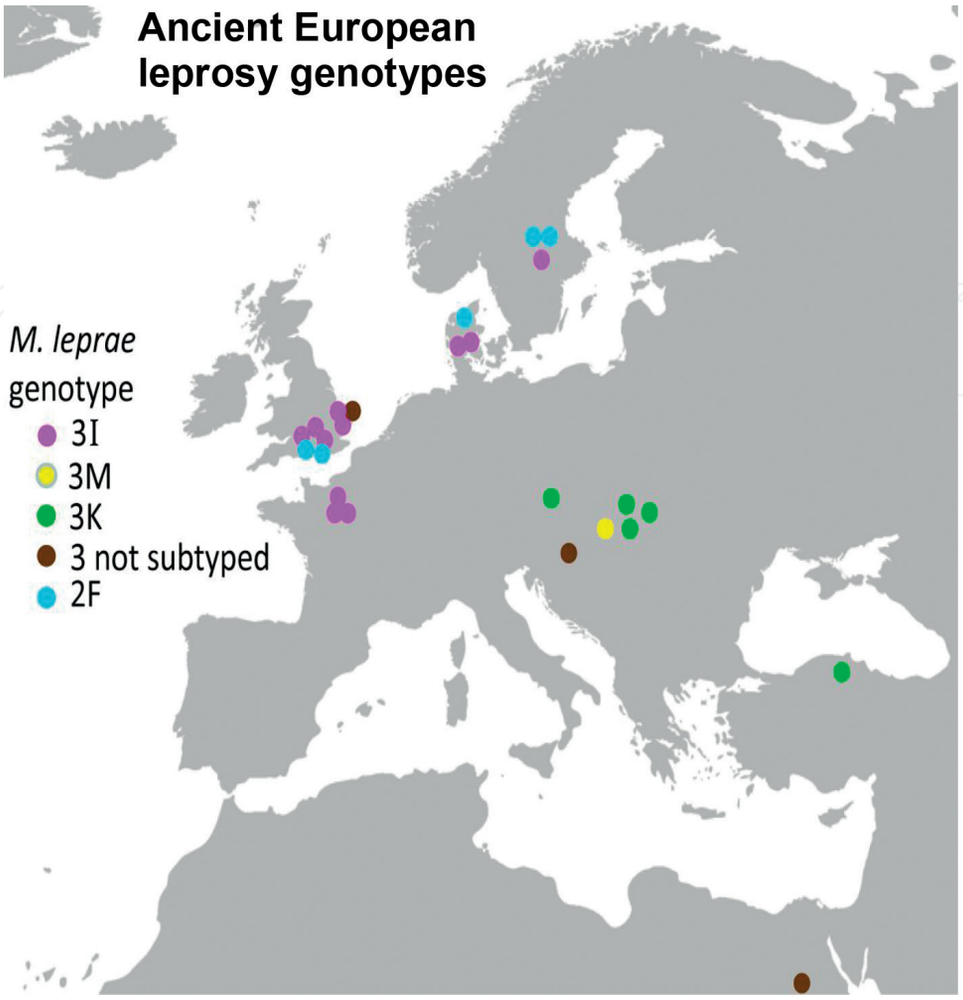

Figure 6. Geographical distribution of ancient leprosy sub-genotypes in the European area. Three type 3 strains are included where sub-typing was not determined.

3.4. Co-infection of leprosy and tuberculosis

Leprosy was a significant problem in Scandinavia until a century ago, leading to the identification of the leprosy bacillus by Hansen in 1871 [95], although publication was delayed due to the inevitable unsuccessful attempts at culture. In Central Europe, however, leprosy was prevalent in the first millennium CE, but a subsequent decline appeared to coincide with the upsurge of tuberculosis. Support for a period of overlap between leprosy and tuberculosis has been provided by a number of clear archaeological examples of dual infection, from first century AD Israel, fourth to fifth century Roman Egypt, seventh to eleventh century Hungary, eighth to ninth century Austria to tenth to thirteenth century Sweden [30, 72]. In one particular case, it was possible to use quantitative lipid biomarker analysis to estimate the relative amount of leprosy and tuberculosis infection [28–30]. Mathematical modelling to explore the epidemiological consequences of dual infection concluded that the disappearance of leprosy could indeed be explained by *M. leprae*/*M. tuberculosis* co-infections [96]. This may explain the present absence of indigenous human leprosy in Europe. Currently characterised *M. leprae*/*M. tuberculosis* co-infections are summarised in **Table 2**.

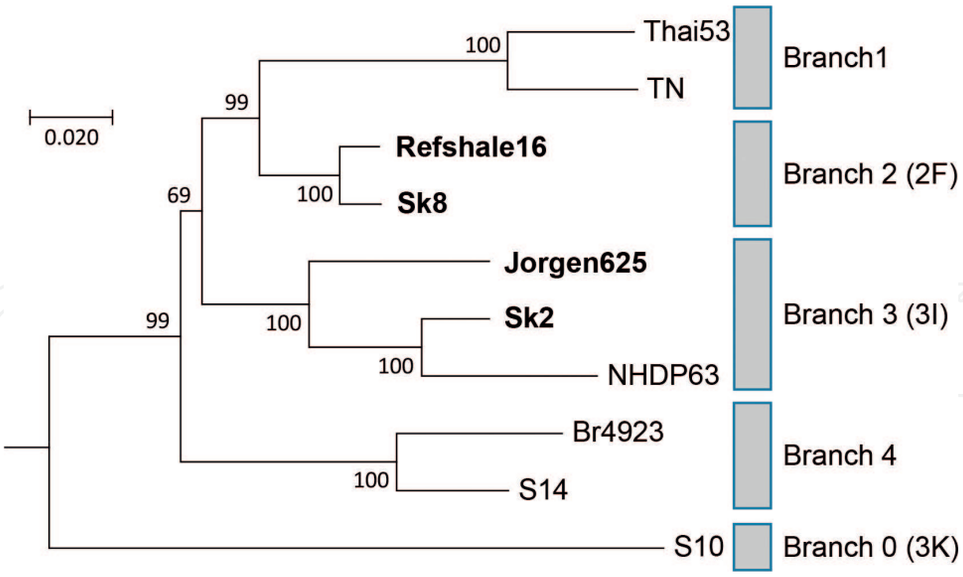

Figure 7. A phylogeny of selected *M. leprae* strains. The phylogeny was derived from an alignment of genomic SNPs [46]; ancient strains are denoted in bold. Phylogenies were generated in MEGA7 [105], using Maximum Likelihood methods. Phylogenies based on Neighbour Joining methods generated similar dendrograms. The scale represents the number of substitutions per site. Bootstrap values were determined from 500 replicates. '*M. lepromatosis*' was used as an out-group (not shown). Subtypes are indicated in brackets.

Authors	Year	Region	Century (CE)	Methods and comments
Nuorala et al. [97]	2004	Sweden	10th–13th	**PCR**: ML RLEP 129/99 bp; MTB 123 bp/92 bp. Nested products sequenced
Donoghue et al. [72]	2005	Egypt	4th–5th	**PCR**: ML RLEP 129/99 bp; MTB 123 bp/92 bp
		Hungary	10th–16th	
		Israel	1st	
		Sweden	10th–13th	
Matheson et al. [69]	2009	Israel	1st	**PCR**: ML RLEP 129/99 bp; MTB IS6110 123/92 bp
Minnikin et al. [29]	2011	Hungary	7th	**PCR**: Not re-tested; **Lipids**: mycolates and mycocerosates indicate relative disease load for Kiskundorozsma-Daruhalom dűlő II Grave KD517
Minnikin et al. [29]	2011	Hungary	15th	**PCR**: Not re-tested; **Lipids**: MTB methoxymycolates and ML ketomycolates for Szombathely Grave 6
Molnár et al. [74]	2015	Hungary	7th–9th	**PCR**: MTB IS6110 123/92 bp; IS1081 113 bp; ML not tested; **Lipids**: mycolates, mycolipenate and mycocerosates for Bélmegyer-Csömöki domb Grave 22
Donoghue et al. [30]	2015	Central and Eastern Europe	Various 6th–11th	**PCR**: ML RLEP 129/99 bp; 111 bp; 80 bp and probe; RepLep 66 bp and probe; SNP typing indicates migratory patterns into Europe. Coinfections suggest role of MTB in decline of European leprosy

Cases are listed according to year of study.

Table 2. aDNA and lipid biomarker detection of ancient ***M. leprae*** and ***M. tuberculosis*** complex co-infections.

4. Origins and evolution of leprosy

4.1. Genomics of modern leprosy

Whole genome sequencing has revealed large numbers of pseudogenes in both *M. leprae* and '*M. lepromatosis*' [7, 8, 98–100]. These genomic studies revealed that both *M. leprae* and '*M. lepromatosis*' have undergone a reductive evolution in which extensive recombination events have occurred between dispersed repetitive sequences, leading to less than half of their genomes containing functional genes. In a preliminary study [7], it was indicated that the genome of '*M. lepromatosis*' (~3.22 Mb) was 1.6% smaller than that (~3.27 Mb) of *M. leprae* [98, 99]. A comprehensive parallel study gave a similar genome size of ~3.21 for '*M. lepromatosis*' [8]. Functional comparisons revealed that whereas *M. leprae* has a defective *heme* pathway, '*M. lepromatosis*' lacked several genes needed for amino acid synthesis [8]. It is apparent that '*M. lepromatosis*' is the closest known mycobacterial taxon to the established species of *M. leprae*. Phylogenetic analysis indicates that '*M. lepromatosis*' and *M. leprae* diverged from a most recent common ancestor (MRCA) about 13.9 million years ago [8].

4.2. Evolutionary origins of leprosy bacilli

The deep origins of mycobacterial disease remain to be clearly defined [3, 47, 98, 99]. In contrast to tuberculosis, which appears to stretch back hundreds of thousands of years [57, 58], the earliest manifestations of human leprosy are found in skeletal remains only about 4000 years old [101]. However, the older participation of animal hosts cannot be ruled out, as it is increasingly evident that Pleistocene megafauna may have had a major involvement in tuberculosis evolution [58]. A possible ancestral organism to the organisms that cause leprosy may have been more like modern *M. haemophilum*, an emerging pathogen with a variety of possible natural reservoirs. The first significant link identified between *M. leprae* and *M. haemophilum* was established a quarter of a century ago in a study of the so-called 'phenolic glycolipids' (PGLs) [13]. As shown in **Figure 3**, the similarity in the oligosaccharide composition of the PGLs was striking and the mycocerosate profile (**Figure 2**) almost identical. This early key observation was subsequently reinforced by taxonomic studies that showed a close association of *M. leprae* and *M. haemophilum* [14, 15, 102]. Again, in studies comparing *M. leprae* and '*M. lepromatosis*', *M. haemophilum* was the nearest neighbour [8, 39], as illustrated in **Figure 8**. The recent determination of a full genome (~4.23 Mb) for *M. haemophilum* confirmed the close link [16], as shown in **Figure 8**. *M. haemophilum* is consistently placed outside of the *M. leprae*/*M. lepromatosis* group but between *M. leprae* and other mycobacteria such as the *M. tuberculosis* complex. It was suggested that the reductive evolution of *M. leprae* and '*M. lepromatosis*' was not shared with the most recent common ancestor but started after the divergence of *M. haemophilum* from both taxa [16]. The relatedness of *M. haemophilum*, *M. leprae*, '*M. lepromatosis*' and related taxa is shown in **Figure 8**.

4.3. Animal and environmental sources of leprosy ancestors

To assess the involvement of ancient relatives of *M. haemophilum* in the evolution of leprosy bacilli, it is necessary to consider the ecological, environmental and animal host preferences of

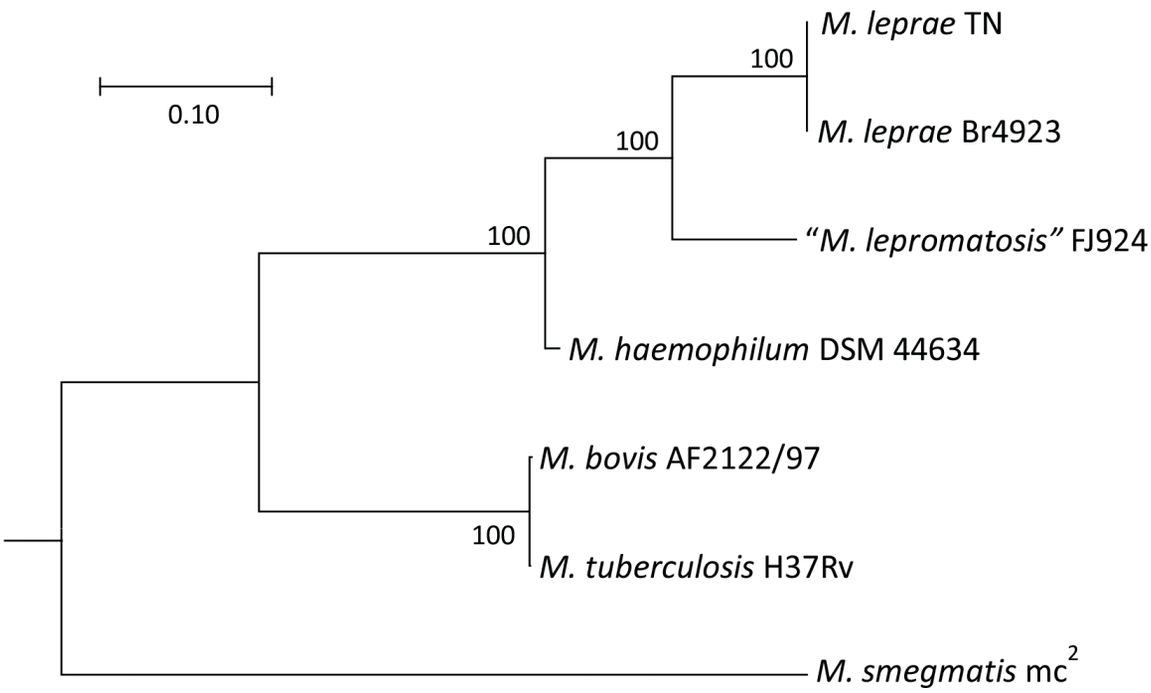

Figure 8. A phylogeny of *M. leprae* strains and other mycobacterial species. Genomic sequence coding for DnaN [103] from illustrative mycobacterial species was aligned with Clustal Omega [104] and their phylogeny inferred with MEGA7 [105] using the Maximum Likelihood methods and the Hasegawa-Kishino-Yano model with possible invariant sites. Phylogenies consistent with this interpretation were obtained with Neighbour-joining methods and when concatenated amino acid sequence of conserved proteins was used in the alignment. Bootstrap values are derived from 500 replicates.

this taxon. *M. haemophilum* is slow growing, requires iron supplementation and prefers a low growth temperature of 30°C. The first description of *M. haemophilum* was as a pathogen causing skin infections, particularly not only in immunocompromised patients [106, 107], but also in healthy children [108]. In a range of children, a variety of other clinical manifestations were encountered [15]. In two instances, *M. haemophilum* infections mimicked the appearance of leprosy [109, 110] and a co-infection of *M. leprae* and *M. haemophilum* has been reported [111]. Also, animal infections are common, with zebra fish (*Danio rerio*) being particularly susceptible [15]. More recently, a heavily infected leatherback sea turtle (*Dermochelys coriacea*) was found [112]. Infection of a haemophiliac with *M. haemophilum* was linked to contact with raw shrimp [113]. This suggests that *M. haemophilum* can move freely in a variety of environments, but it does not give a clear indication whether there is a particular zoonotic host in which the evolution of *M. haemophilum* may have occurred.

As noted previously, both *M. leprae* and '*M. lepromatosis*' can cause disease in squirrels [11, 12, 43, 44]. The presence of leprosy in armadillos is long established [9, 10, 114, 115] and, indeed, the armadillo was a prime source of material for early studies of the leprosy bacillus [79–81, 83, 84]. It is apparent that infected armadillos can spread leprosy to the human population [9, 10]. However, the leprosy introduced into the Americas by human migration was passed on to indigenous armadillos [46] so they can be eliminated as an environmental evolutionary source. The involvement of squirrels in the UK is more intriguing as it is difficult to envisage how

the diseases can have been contracted from human sources. A direct evolutionary pathway from ancient squirrel-like animals to humans is unlikely, but it is possible that squirrels are representative of other animals that may have acted as environmental reservoirs. In the case of '*M. lepromatosis*', a geographical association between patients and Mexican field rats (*Rattus rattus*) suggests a possible environmental reservoir [8].

4.4. Animal diseases resembling leprosy

Cases of tuberculoid nodular thelitis in both cattle [116] and goats [117] appear to be caused by uncultivable acid-fast species related to *M. leprae* and '*M. lepromatosis*'. However, the interrelationships between these agents, infecting cattle and goats, need to be defined more precisely before the disease can be considered as a true variety of leprosy. A complex scenario is emerging regarding the status of infections categorised as 'feline leprosy' [118–121]. After many early reports of diverse manifestations of cat leprosy, a definitive study clarified the scene [122]. It was apparent that the rat leprosy bacillus, *M. lepraemurium*, made a contribution to disease, but the influence of a novel uncultivable *Mycobacterium*, whose closest relative was *M. malmoense*, was noted. In a follow-up study [123], it was observed that younger cats were susceptible to *M. lepraemurium*, but more mature felines typically harboured the novel uncultivable agent. In an interesting development, PCR amplification of 16S rRNA sequences, from the uncultured feline agent AJ294740-6, showed that the greatest nucleotide identity was shared with *M. leprae* and *M. haemophilum*, as well as *M. malmoense*; indeed a specific additional nucleotide correlated with only with *M. leprae* [124]. This particular taxon, expressed in cases from eastern Australia, New Zealand and possibly Canada, has been provisionally labelled '*M. lepraefelis*' [121]. Three North American feline infections appeared to be caused by another uncultivable agent with close 16S rRNA relatedness to *M. leprae* and more distant affinity to *M. haemophilum,* among other species [125]. Initially labelled '*M. visibilis*', but more properly '*M. visibile*', this taxon remains uncharacterised and unfortunately unavailable for further study [120]. In a limited area of southeast Australia, studies of feline leprosy have revealed the presence of *M. lepraemurium* and an uncultivable novel agent, labelled '*M. tarwinense*'. This agent was indicated to be a fastidious member of the *M. simiae* complex [120, 126] so it does not appear to have a direct relationship with *M. leprae* or '*M. lepromatosis*'.

4.5. Overall interrelationships of leprosy affiliates

The precise interrelationships between all the bacterial taxa causing leprosy-like diseases require further study. It is clear that *M. leprae* or '*M. lepromatosis*' cause human leprosy and the same agents can routinely infect armadillos and squirrels. The apparent affinities of the feline leprosy taxon, labelled '*M. lepraefelis*', with *M. leprae* and *M. haemophilum* must be explored. The agents causing tuberculoid nodular thelitis in cattle and goats appear to have an affinity with established leprosy bacilli and this should be thoroughly investigated. In view of present uncertainties, it is premature to consider any concept of an *M. leprae* complex, as has been discussed [6, 8, 118, 127].

The possible origins and interrelationships of all agents causing leprosy-like disease are summarised in **Figure 9**. The phylogeny of *M. haemophilum* with *M. leprae* and '*M. lepromatosis*' indicates a deep common ancestor for all three taxa [16]; this ancestor is provisionally labelled

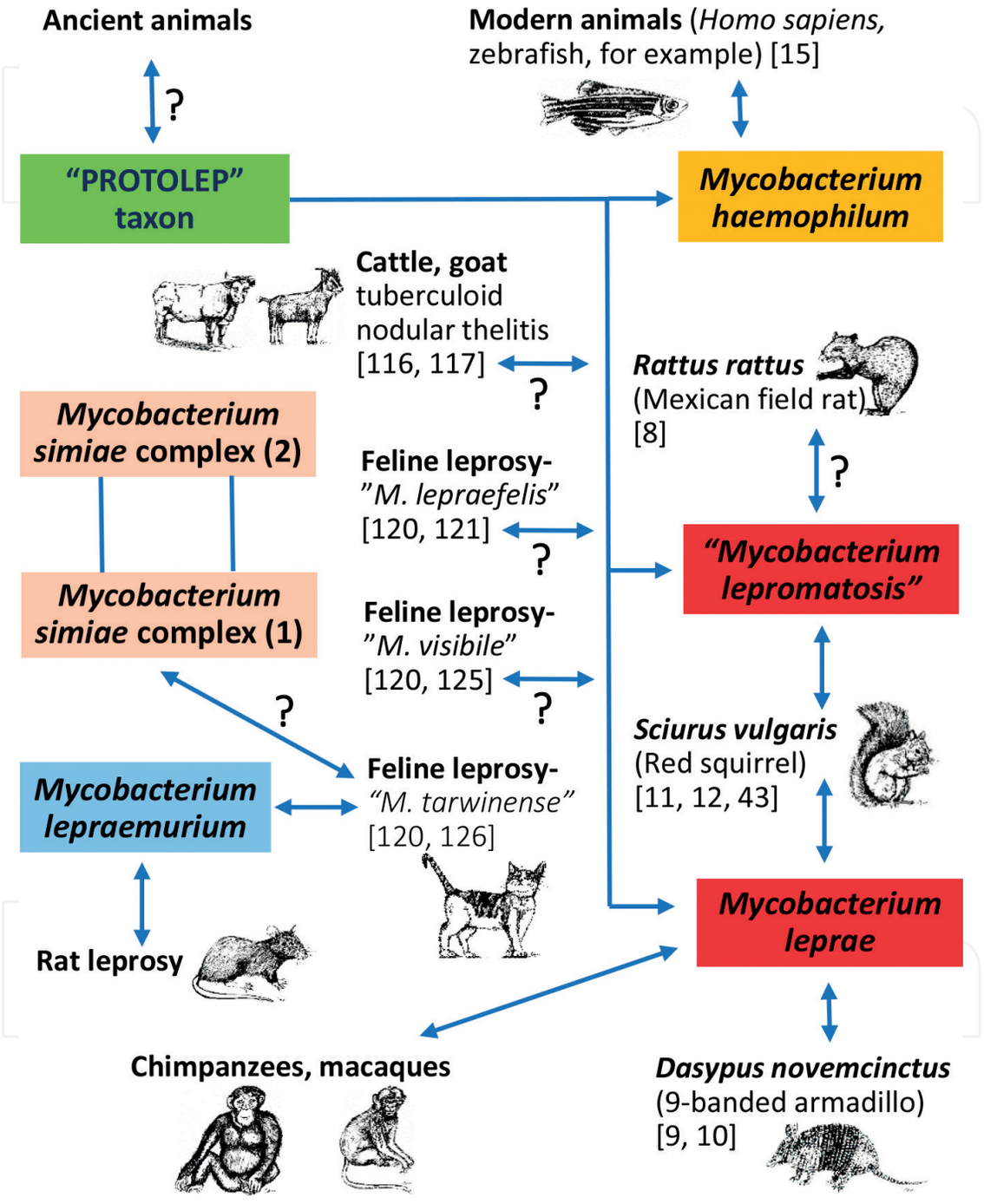

Figure 9. Origins and interrelationships of agents causing leprosy-like disease. Proposed relationships requiring further study are indicated (?). 'PROTOLEP' represents a prototype taxon with the specific type of outer membrane lipids expressed in *M. haemophilum, M. leprae* and possibly '*M. lepromatosis*'. *M. simiae* complex (**1**) represents species (*M. florentinum, M. interjectum, M. sherrissii, M. triplex*) apparently expressing genes for PDIM synthesis; *M. simiae* complex (**2**) includes the remaining species [128].

'PROTOLEP' in **Figure 9**. This hypothetical taxon is considered to incorporate characteristic cell envelope lipids, such as the C_{34} mycocerosates found in *M. leprae* and *M. haemophilum* (**Figure 2**). Sensitive lipid biomarker analysis has the potential to help identify the uncultivable agents causing feline leprosy ('*M. lepraefelis*', '*M. visibile*') and tuberculoid nodular thelitis in cattle and goats (**Figure 9**). It is an open question whether these agents have any affinity with *M. leprae*, '*M. lepromatosis*' or *M. haemophilum*, but it seems likely that the feline cases that are associated with both *M. lepraemurium* and '*M. tarwinense*' [119, 120, 126] (**Figure 9**) are distinct. '*M. tarwinense*' appears to be an affiliate of the *M. simiae* complex, which appeared to have little phylogeny with *M. leprae* and related taxa until detailed genomic characterisation of nontuberculous mycobacteria indicated that particular *M. simiae* complex members (*M. florentinum*, *M. interjectum*, *M. sherrissii*, *M. triplex*) apparently have genes for PDIM synthesis (**Figure 9**) [128]. It would be of interest to discover if there is any similarity between the proven PDIMs of *M. leprae* and those suggested to be expressed by these members of the *M. simiae* complex.

5. Conclusions

An understanding of the origins and spread of leprosy depends on establishing detailed knowledge of the ancient genotypes and their correlation with modern disease. The overall scenario has been expanded by the recent characterisation of the distinct modern clade, currently labelled '*M. lepromatosis*'. The availability of a full genome for '*M. lepromatosis*' is allowing specific probes to be developed to search for ancient expression of this biotype. Ongoing research is demonstrating that subtle lipid biomarker differences may be of value in distinguishing '*M. lepromatosis*' from *M. leprae*. The overall picture for the global development of leprosy suggests that the ancient disease evolved into a number of recognisable clades in Africa/Eurasia. It is clear that leprosy was introduced into the Americas by human migration, and the disease was passed on to indigenous armadillos. The deeper origins of leprosy appear to be inextricably linked to relatives of the environmental taxon *M. haemophilum*. Diseases in cats, cattle and goats, with affiliations and resemblances to leprosy, require detailed investigation.

Acknowledgements

For basic studies on leprosy and related mycobacteria, DEM and GSB are grateful for support from LEPRA (www.lepra.org.uk), in the form of Studentships: Gary Dobson 1980–1983, Gurdyal S. Besra 1987–1990, Susanne Hartmann 1991–1994. The Leverhulme Trust Project Grant F/00 094/BL supported the development of lipid biomarker detection of ancient leprosy (OY-CL, DEM, GSB). GSB acknowledges support in the form of a Personal Research Chair from Mr. James Bardrick and the UK Medical Research Council (grant MR/K012118/1) and Wellcome Trust (grant 081569/Z/06/Z).

Author details

Helen D. Donoghue[1]*, G. Michael Taylor[2], Tom A. Mendum[2], Graham R. Stewart[2], Leen Rigouts[3], Oona Y-C. Lee[4], Houdini H.T. Wu[4], Gurdyal S. Besra[4] and David E. Minnikin[4]

*Address all correspondence to: h.donoghue@ucl.ac.uk

1 Centre for Clinical Microbiology, University College London, London, UK

2 Department of Microbial Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK

3 Mycobacteriology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

4 Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, UK

References

[1] Han XY, Seo Y-H, Sizer KC, Schoberle T, May GS, Spencer JS, Li W, Nair RG. A new *Mycobacterium* species causing diffuse lepromatous leprosy. American Journal of Clinical Pathology. 2008;**130**:856-864. DOI: 10.1309/AJCPP72FJZZRRVMM

[2] Kowalska M, Kowalik A. *Mycobacterium leprae*: Pathogenic agent in leprosy. Discovery of new species "*Mycobacterium lepromatosis*". Perspectives in research and diagnosis of leprosy. International Maritime Health. 2012;**63**:213-218

[3] Han XY, Silva FJ. On the age of leprosy. PLoS Neglected Tropical Diseases. 2014;**8**:e2544. DOI: 10.1371/journal.pntd.0002544

[4] Han XY, Aung FM, Choon SE, Werner B. Analysis of the leprosy agents *Mycobacterium leprae* and "*Mycobacterium lepromatosis*" in four countries. American Journal of Clinical Pathology. 2014;**142**:524-532. DOI: 10.1309/AJCP1GLCBE5CDZRM

[5] Sotiriou MC, Stryjewska BM, Hill C. Case report: Two cases of leprosy in siblings caused by *Mycobacterium lepromatosis* and review of the literature. The American Journal of Tropical Medicine and Hygiene. 2016;**95**:522-527. DOI: 10.4269/ajtmh.16-0076

[6] Scollard DM. Infection with "*Mycobacterium lepromatosis*". The American Journal of Tropical Medicine and Hygiene. 2016;**95**:500-501. DOI: 10.4269/ajtmh.16-0473

[7] Han XY, Mistry NA, Thompson EJ, Tang H-L, Khanna K, Zhang L. Draft genome sequence of new leprosy agent "*Mycobacterium lepromatosis*". Genome Announcements. 2015;**3**:e00513-15. DOI: 10.1128/genomeA.00513-15

[8] Singh P, Benjak A, Schuenemann VJ, Herbig A, Avanzi C, Busso P, Nieselt K, Krause J, Vera-Cabrera L, Cole ST. Insight into the evolution and origin of leprosy bacilli from the genome sequence of "*Mycobacterium lepromatosis*". PNAS. 2015;**112**:4459-4464. DOI: www.pnas.org/cgi/doi/10.1073/pnas.1421504112

[9] Truman RW, Singh P, Sharma R, Busso P, Rougemont J, Paniz-Mondolfi A, Kapopoulou A, Brisse S, Scollard DM, Gillis GP, Cole ST. Probable zoonotic leprosy in the southern United States. New England Journal of Medicine. 2011;**364**:1626-1633

[10] Truman RW, Ebenezer GJ, Pena MT, Sharma R, Balamayooran G, Gillingwater TH, Scollard DM, McArthur JC, Rambukkana A. The armadillo as a model for peripheral neuropathy in leprosy. ILAR Journal. 2014;**54**:304-314. DOI: 10.1093/ilar/ilt050

[11] Meredith A, Del Pozo J, Smith S, Milne E. Leprosy in red squirrels in Scotland. The Veterinary Record. 2014;**20**:285-286. DOI: 10.1136/vr.g5680

[12] Avanzi C, del-Pozo J, Benjak A, Stevenson K, Simpson VR, Busso P, McLuckie J, Loiseau C, Lawton C, Schoening J, Shaw DJ, Piton J, Vera-Cabrera L, Velarde-Felix JS, McDermott F, Gordon SV, Cole SY, Meredith AL. Red squirrels in the British Isles are infected with leprosy bacilli. Science. 2016;**354**:743-746. DOI: 10.1126/science.aah3783

[13] Besra GS, McNeil M, Minnikin DE, Portaels F, Ridell M, Brennan PJ. Structural elucidation and antigenicity of a novel phenolic glycolipid antigen from *Mycobacterium haemophilum*. Biochemistry. 1991;**30**:7772-7777

[14] Harmsen D, Dostal S, Roth A, Niemann S, Rothgänger J, Sammeth M, Albert J, Frosch M, Richter E. RIDOM: Comprehensive and public sequence database for identification of mycobacterial species. BMC Infectious Diseases. 2003;**3**:26 http://www.biomedcentral.com/1471-2334/3/26

[15] Lindeboom JA, Bruijnesteijn van Coppenraet LES, van Soolingen D, Prins JM, Kuijper EJ. Clinical manifestations, diagnosis, and treatment of *Mycobacterium haemophilum* infections. Clinical Microbiology Reviews. 2011;**24**:701-717. DOI: 10.1128/CMR.00020-11

[16] Tufariello JM, Kerantzas CA, Vilchèze C, Calder RB, Nordberg EK, Fischer JA, Hartman TE, Yang E, Driscoll T, Cole LE, Sebra R, Maqbool SB, Wattam AR, Jacobs WR Jr. The complete genome sequence of the emerging pathogen *Mycobacterium haemophilum* explains its unique culture requirements. MBio. 2015;**6**:e01313-15. DOI: 10.1128/mBio.01313-15

[17] Møller-Christensen V, Weiss DL. One of the oldest datable skeletons with leprous bone-changes from the Naestved Leprosy Hospital churchyard in Denmark. International Journal of Leprosy and Other Mycobacterial Diseases. 1971;**39**:172-182

[18] Ortner DJ, Putschar WGJ. Identification of pathological conditions in human skeletal remains. Smithsonian Contributions to Anthropology. 1981;**28**:167-169

[19] Danielsen K. *Odontodysplasia leprosa* in Danish medieval skeletons. Tandlaegebladet. 1970;**74**:603-625

[20] Matos VMJ, Santos AL. *Leprogenic odontodysplasia*: New evidence from the St. Jørgen's medieval leprosarium cemetery (Odense, Denmark). Anthropological Science. 2013;**121**:43-47

[21] Taylor GM, Tucker K, Butler R, Pike AWG, Lewis J, Roffey S, Marter P, Lee OY-C, HHT W, Minnikin DE, Besra GS, Singh P, Cole ST, Stewart GR. Detection and strain typing of ancient *Mycobacterium leprae* from a medieval leprosy hospital. PLoS ONE. 2013;**8**:e62406. DOI: 10.1371/journal.pone.0062406

[22] Rogers J, Waldron T. Infections in palaeopathology: The basis of classification according to most probable cause. Journal of Archaeological Science. 1989;**16**:621-625

[23] Rafi A, Spigelman M, Stanford J, Lemma E, Donoghue H, Zias J. DNA of *Mycobacterium leprae* detected by PCR in an ancient bone. International Journal of Osteoarchaeology. 1994;**4**:287-290

[24] Donoghue HD, Gladykowska-Rzeczycka J, Marcsik A, Holton J, Spigelman M. *Mycobacterium leprae* in archaeological samples. In: Roberts CA, Lewis ME, Manchester K, editors. The Past and Present of Leprosy: Archaeological, Historical, Palaeopathological and Clinical Approaches. British Archaeological Reports Series. Oxford: Archaeopress; 2002. pp. 271-285. ISBN: 1 84171 434 8

[25] Taylor GM, Watson CL, Bouwman AS, Lockwood DNJ, Mays SA. Variable nucleotide tandem repeat (VNTR) typing of two palaeopathological cases of lepromatous leprosy from Mediaeval England. Journal of Archaeological Science. 2006;**33**:1569-1579. DOI: 10.1016/j.jas.2006.02.008

[26] Monot M, Honoré N, Garnier T, Zidane N, Sherafi D, Paniz-Mondolfi A, Matsuoka M, Taylor GM, Donoghue HD, Bouwman A, Mays S, Watson C, Lockwood D, Khamispour A, Dowlati Y, Jianping S, Rea TH, Vera-Cabrera L, Stefani MM, Banu S, MacDonald M, Sapkota BR, Spencer JS, Thomas J, Hasrshman K, Singh P, Busso P, Gattiker A, Rougemont J, Brennan PJ, Cole ST. Comparative genomic and phylogeographic analysis of *Mycobacterium leprae*. Nature Genetics. 2009;**41**:1282-1289. DOI: 10.1038/ng.477

[27] Taylor GM, Blau S, Mays S, Monot M, Lee OY-C, Minnikin DE, Besra GS, Cole ST, Rutland P. *Mycobacterium leprae* genotype amplified from an archaeological case of lepromatous leprosy in Central Asia. Journal of Archaeological Science. 2009;**36**:2408-2414. DOI: 10.1016/j.jas.2009.06.026

[28] Lee OY-C, Bull ID, Molnár E, Marcsik A, Pálfi G, Donoghue HD, Besra GS, Minnikin DE. Integrated strategies for the use of lipid biomarkers in the diagnosis of ancient mycobacterial disease. In: Mitchell PD, Buckberry J, editors. Proceedings of the Twelfth Annual Conference of the British Association for Biological Anthropology and Osteoarchaeology. BAR International Series 2380. Oxford: Archaeopress; 2012. pp. 63-69. ISBN: 978-1-4073-0970-5

[29] Minnikin DE, Besra GS, Lee OY-C, Spigelman M, Donoghue HD. 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, editors. Yearbook of Mummy Studies. Vol. 1. Munich, Germany: Verlag Dr. Friedrich Pfeil; 2011. pp. 109-114

[30] Donoghue HD, Taylor GM, Marcsik A, Molnár E, Pálfi G, Pap I, Teschler-Nicola M, Pinhasi R, Erdal YS, Velemínsky P, Likovsky J, Belcastro MG, Mariotti V, Riga A, Rubini M, Zaio P, Besra GS, Lee OY-C, Wu HHT, Minnikin DE, Bull ID, O'Grady J, Spigelman M. A migration-driven model for the historical spread of leprosy in medieval Eastern and Central Europe. Infection, Genetics and Evolution. 2015;**31**:250-256. DOI: 10.1016/j.meegid.2015.02.001

[31] World Health Organisation. Leprosy fact sheet. 2017, updated February 2017. Available from: http://www.who.int/mediacentre/factsheets/fs101/en/ [Accessed: 2017-09-26]

[32] Britton WJ, DNJ L. Leprosy. Lancet. 2004;**36**:209-1219

[33] Araujo S, Freitas LO, Goulart LR, Bernardes Goulart IM. Molecular evidence for the aerial route of infection of *Mycobacterium leprae* and the role of asymptomatic carriers in the persistence of leprosy. Clinical Infectious Diseases. 2016;**63**:412-420. DOI: 10.1093/cid/ciw570

[34] Ridley DS. Histological classification and the immunological spectrum of leprosy. Bulletin of the World Health Organization. 1974;**51**:451-465

[35] World Health Organization. Classification of leprosy. 2017. Available from: http://www.who.int/lep/classification/en/. [Accessed: 2017-09-26]

[36] Santos VS, Santos LC, Lôbo LVR, Lemos LMD, Gurgel RQ, Cuevas LE. Leprosy and disability in children younger than 15 years in an endemic area of Northeast Brazil. The Pediatric Infectious Disease Journal. 2015;**34**:e44-e47. DOI: 10.1097/INF.0000000000000592

[37] Talhari C, Talhari S, Penna GO. Clinical aspects of leprosy. Clinics in Dermatology. 2015;**33**:26-37 http://dx.doi.org/10.1016/j.clindermatol.2014.07.002

[38] Rodrigues LC, Lockwood DNJ. Leprosy now: Epidemiology, progress, challenges, and research gaps. The Lancet Infectious Diseases. 2011;**11**:464-470

[39] Han XY, Sizer KC, Thompson EJ, Kabanja J, Li J, Hu P, Gómez-Valero L, Silva FJ. Comparative sequence analysis of *Mycobacterium leprae* and the new leprosy-causing "*Mycobacterium lepromatosis*". Journal of Bacteriology. 2009;**191**:6067-6074

[40] Vera-Cabrera L, Escalante-Fuentes WG, Gomez-Flores M, Ocampo-Candiani J, Busso P, Singh P, Cole ST. Case of diffuse lepromatous leprosy associated with "*Mycobacterium lepromatosis*". Journal of Clinical Microbiology. 2011;**49**:4366-4368. DOI: 10.1128/JCM.05634-11

[41] Vera-Cabrera L, Escalante-Fuentes W, Ocampo-Garza SS, Ocampo-Candiani J, Molina-Torres CA, Avanzi C, Benjak A, Busso P, Singh P, Cole ST. *Mycobacterium lepromatosis* infections in Nuevo León, Mexico. Journal of Clinical Microbiology. 2015;**53**:1945-1946. DOI: 10.1128/JCM.03667-14

[42] Velarde-Félix JS, Alvarado-Villa G, Vera-Cabrera L. "Lucio's phenomenon" associated with *Mycobacterium lepromatosis*. The American Journal of Tropical Medicine and Hygiene. 2016;**94**:483-484. DOI: 10.4269/ajtmh.15-0439

[43] Simpson V, Hargreaves J, Butler H, Blackett T, Stevenson K, McLuckie J. Leprosy in red squirrels on the Isle of Wight and Brownsea Island. The Veterinary Record. 2015;**177**:206-207. DOI: 10.1136/vr.h4491

[44] Butler HM, Stevenson K, McLuckie J, Simpson V. Further evidence of leprosy in Isle of Wight red squirrels. The Veterinary Record. 2017;**180**:407

[45] Stinear TP, Brosch R. Leprosy in red squirrels. Science. 2016;**354**:702-703. DOI: 10.1126/science.aal0145

[46] Schuenemann VJ, Singh P, Mendum TA, Krause-Kyora B, Jäger G, Bos KI, Herbig A, Economou C, Benjak A, Busso P, Nebel A, Boldsen JL, Kjellström A, Wu H, Stewart GR, Taylor GM, Bauer P, Lee OY-C, Wu HHT, Minnikin DE, Besra GS, Tucker K, Roffey S, Sow SO, Cole ST, Nieselt K, Krause J. Genome-wide comparison of medieval and modern *Mycobacterium leprae*. Science. 2013;**341**:179-183. DOI: 10.1126/science.1238286

[47] Monot M, Honoré N, Garnier T, Araoz R, Coppée J-Y, Lacroix C, Sow S, Spencer JS, Truman RW, Williams DL, Gelber R, Virmond M, Flageul B, Cho S-N, Ji B, Paniz-Mondolfi A, Convit J, Young S, Fine PE, Rasolofo V, Brennan PJ, Cole ST. On the origin of leprosy. Science. 2005;**308**:1040-1042. DOI: 10.1126/science/1109759

[48] Maiden MCJ. Putting leprosy on the map. Nature Genetics. 2009;**41**:1264-1266

[49] Watson CL, Popescu E, Boldsen J, Slaus M, Lockwood DNJ. Single nucleotide polymorphism analysis of European archaeological *M. leprae* DNA. PLoS One. 2009;**4**:e7547. DOI: 10.1371/journal.pone.0007547

[50] Economou C, Kjellström A, Lidén K, Panagopoulos I. Ancient-DNA reveals an Asian type of *Mycobacterium leprae* in medieval Scandinavia. Journal of Archaeological Science. 2013;**40**:465-470. DOI: 10.1016/j.jas.2012.07.005

[51] Economou C, Kjellström A, Lidén K, Panagopoulos I. Corrigendum to "Ancient-DNA reveals an Asian type of *Mycobacterium leprae* in medieval Scandinavia" [J. Archaeol. Sci. 40 (1) (2013) 465-470]. Journal of Archaeological Science. 2013;**40**:2867. DOI: 10.1016/j.jas.2013.03.008

[52] Suzuki K, Takigawa W, Tanigawa K, Nakamura K, Ishido Y, Kawashima A, Wu H, Akama T, Sue M, Yoshihara A, Mori S, Ishii N. Detection of *Mycobacterium leprae* DNA from archaeological skeletal remains in Japan using whole genome amplification and polymerase chain reaction. PLoS One. 2010;**5**:e12422. DOI: 10.1371/journal.pone.0012422

[53] Mendum TA, Schuenemann VJ, Roffrey S, Taylor GM, Wu H, Singh P, Tucker K, Hinds J, Cole ST, Kierzek AM, Nieselt K, Krause J, Stewart GR. *Mycobacterium leprae* genomes from a British medieval leprosy hospital: Towards understanding an ancient epidemic. BMC Genomics. 2014;**15**:270. http://www.biomedcentral.com/1471-2164/15/270

[54] Yuan Y, Wen Y, You Y, Xing Y, Li H, Weng X, Wu N, Liu S, Zhang S, Zhang W, Zhang Y. Characterization of *Mycobacterium leprae* genotypes in China—Identification of a new polymorphism C251T in the 16S rRNA gene. PLoS One. 2015;**10**:e0133268. DOI: 10.1371/journal.pone.0133268

[55] Cardona-Castro N, Cortés E, Beltrán C, Romero M, Badel-Mogolión JE, Bedoya G. Human genetic ancestral composition correlates with the origin of *Mycobacterium leprae* strains in a leprosy endemic population. PLoS Neglected Tropical Diseases. 2015;**9**:e0004045. DOI: 10.1371/journal.pntd.0004045

[56] Walsh GP, Meyers WM, Binford CH. Naturally acquired leprosy in the nine-banded armadillo: A decade of experience 1975-1985. Journal of Leukocyte Biology. 1986;**40**:645-656

[57] Minnikin DE, Lee OY-C, HHT W, Nataraj V, Donoghue HD, Ridell M, Watanabe M, Alderwick L, Bhatt A, Besra GS. Pathophysiological implications of cell envelope structure in *Mycobacterium tuberculosis* and related taxa. In: Ribón W, editor. Tuberculosis—Expanding Knowledge. Rijeka: InTech Open Access Publisher; 2015. pp. 146-175. ISBN: 978-953-51-4194-5. DOI: 10.5772/59585

[58] Jankute M, Nataraj V, Lee OY-C, Wu HHT, Ridell M, Garton NJ, Barer MR, Minnikin DE, Bhatt A, Besra GS. The role of hydrophobicity in tuberculosis evolution and pathogenicity. Scientific Reports. 2017;**7**:1315. DOI: 10.1038/s41598-017-01501-0

[59] Silva CAM, Danelishvili L, McNamara M, Berredo-Pinho M, Bildfell R, Biet F, Rodrigues LS, Oliveira AV, Bermudez LE, Pessolania MCV. Interaction of *Mycobacterium leprae* with human airway epithelial cells: Adherence, entry, survival and identification of potential adhesins by surface proteome analysis. Infection and Immunity. 2013;**81**:2645-2659. DOI: 10.1128/IAI.00147-13

[60] Kragelund C, Remesova Z, Nielsen JL, Thomsen TR, Eales K, Seviour R, Wanner J, Nielsen PR. Ecophysiology of mycolic acid-containing Actinobacteria (Mycolata) in activated sludge foams. FEMS Microbiology Ecology. 2007;**61**:174-184

[61] Spigelman M, Rubini M. Paleomicrobiology of leprosy. Microbiology Spectrum. 2016;**4**: PoH-0009-2015. DOI: 10.1128/microbiolspec.PoH-0009-2015

[62] Mariotti V, Dutour O, Belcastro MG, Facchini F, Brasili P. Probable early presence of leprosy in Europe in a Celtic skeleton of the 4th–3rd century BC (Casalecchio di Reno, Bologna, Italy). International Journal of Osteoarchaeology. 2005;**15**:311-325. DOI: 10.1002/oa.775

[63] Rubini M, Zaio P, Roberts C. Tuberculosis and leprosy in Italy. New skeletal evidence. HOMO—Journal of Comparative Human Biology. 2014;**65**:13-32. DOI: 10.1016/j.jchb.2013.07.006

[64] Rubini M, Erdal YS, Spigelman M, Zaio P, Donoghue HD. Paleopathological and molecular study on two cases of ancient childhood leprosy from the Roman and Byzantine empires. International Journal of Osteoarchaeology. 2014;**24**:520-582. DOI: 10.1002/oa.2242

[65] Haas CJ, Zink A, Pálfi G, Szeimies U, Nerlich AG. Detection of leprosy in ancient human skeletal remains by molecular identification of *Mycobacterium leprae*. American Journal of Clinical Pathology. 2000;**114**:428-436

[66] Taylor GM, Widdison S, Brown IN, Young D, Molleson T. A mediaeval case of lepromatous leprosy from 13-14th century Orkney, Scotland. Journal of Archaeological Science. 2000;**27**:1133-1138. DOI: 10.1006/jasc.1999.0532

[67] Spigelman M, Donoghue HD. Brief communication: Unusual pathological condition in the lower extremities of a skeleton from ancient Israel. American Journal of Physical Anthropology. 2001;**114**:92-98

[68] Marcsik A, Molnár E, Ősz B, Donoghue H, Zink A, Pálfi G. Adatok a lepra, tuberculosis és syphilis Magyarországi paleopatológiájához. [Paleopathology of leprosy, tuberculosis and syphilis in Hungary]. Folia Anthropology. 2009;**8**:5-34

[69] Matheson CD, Vernon KK, Lahti A, Fratpietro R, Spigelman M, Gibson S, Greenblatt CL, Donoghue HD. Molecular exploration of the first-century *Tomb of the Shroud* in Akeldama, Jerusalem. PLoS One. 2009;**4**:e8319. DOI: 10.1371/journal.pone.0008319

[70] Donoghue HD, Holton J, Spigelman M. PCR primers that can detect low levels of *Mycobacterium leprae* DNA. Journal of Medical Microbiology. 2001;**50**:177-182

[71] Taylor GM, Donoghue HD. Multiple loci variable number tandem repeat (VNTR) analysis (MLVA) of *Mycobacterium leprae* isolates amplified from European archaeological human remains with lepromatous leprosy. Microbes and Infection. 2011;**13**:923-929. DOI: 10.1016/j.micinf.2011.05.003

[72] Donoghue HD, Marcsik A, Matheson C, Vernon K, Nuorala E, Molto JE, Greenblatt CL, Spigelman M. Co-infection of *Mycobacterium tuberculosis* and *Mycobacterium leprae* in human archaeological samples: A possible explanation for the historical decline of leprosy. Philosophical Transactions of the Royal Society B: Biological Sciences. 2005;**272**:389-394

[73] Inskip SA, Taylor GM, Zakrzewski SR, Mays SA, Pike AWG, Llewellyn G, Williams CM, Lee OY-C, Wu HHT, Minnikin DE, Besra GS, Stewart GR. Osteological, biomolecular and geochemical examination of an early Anglo-Saxon case of lepromatous leprosy. PLoS One. 2015;**10**:e0124282. DOI: 10.1371/journal.pone.0124282

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

[75] Roffey S, Tucker K, Filipek-Ogden K, Montgomery J, Cameron J, O'Connell T, Evans J, Marter P, Taylor GM. Investigation of a medieval pilgrim burial excavated from the *Leprosarium* of St Mary Magdalen Winchester, UK. PLOS Neglected Tropical Diseases. 2017;**11**:e0005186. DOI: 10.1371/journal.pntd.0005186

[76] Montiel R, García C, Cañadas MP, Isidro A, Guijo JM, Malgosa A. DNA sequences of *Mycobacterium leprae* recovered from ancient bones. FEMS Microbiology Letters. 2003;**226**:413-414

[77] Likovsky J, Urbanova M, Hajek M, Cerny V, Cech P. Two cases of leprosy from Žatec (Bohemia), dated to the turn of the 12th century and confirmed by DNA analysis for *Mycobacterium leprae*. Journal of Archaeological Science. 2006;**33**:1276-1283

[78] Witas HW, Donoghue HD, Kubiak D, Lewandowska M, Gładykowska-Rzeczycka JJ. Molecular studies on ancient *M. tuberculosis* and *M. leprae*: Methods of pathogen and host DNA analysis. European Journal of Clinical Microbiology & Infectious Diseases. 2015;**34**:1733-1749. DOI: https://doi.org/10.1007/s10096-015-2427-5

[79] Draper P, Dobson G, Minnikin DE, Minnikin SM. The mycolic acids of *Mycobacterium leprae* harvested from experimentally infected nine-banded armadillos. Annals of Microbiology. 1982;**133**:39-47

[80] Minnikin DE, Dobson G, Goodfellow M, Draper P, Magnusson M. Quantitative comparison of the mycolic and fatty acid composition of *Mycobacterium leprae* and *Mycobacterium gordonae*. Journal of General Microbiology. 1985;**131**:2013-2021

[81] Draper P, Payne SN, Dobson G, Minnikin DE. Isolation of a characteristic phthiocerol dimycocerosate from *Mycobacterium leprae*. Journal of General Microbiology. 1983;**129**:859-863

[82] Hunter SW, Brennan PJ. Further specific extracellular phenolic glycolipid antigens and a related diacylphthiocerol from *Mycobacterium leprae*. The Journal of Biological Chemistry. 1983;**258**:7556-7562

[83] Minnikin DE, Dobson G, Draper P. The free lipids of *Mycobacterium leprae* harvested from experimentally infected nine-banded armadillos. Journal of General Microbiology. 1985;**131**:2007-2011

[84] Hunter SW, Brennan PJ. A novel phenolic glycolipid from *Mycobacterium leprae* possibly involved in immunogenicity and pathogenicity. Journal of Bacteriology. 1981;**147**:728-735

[85] Hunter SW, Fujiwara T, Brennan PJ. Structure and antigenicity of the major specific glycolipid antigen of *Mycobacterium leprae*. The Journal of Biological Chemistry. 1982;**257**:15072-15078

[86] Portaels F, Dawson DJ, Larsson L, Rigouts L. Biochemical properties and fatty acid composition of *Mycobacterium haemophilum*: Study of 16 isolates from Australian patients. Journal of Clinical Microbiology. 1993;**31**:26-30

[87] Damato JJ, Collins MT. Radiometric studies with gas-liquid and thin-layer chromatography for rapid demonstration of hemin dependence and characterization of *Mycobacterium haemophilum*. Journal of Clinical Microbiology. 1984;**20**:515-518

[88] Kusaka T, Izumi S. Gas chromatography of constitutive fatty acids in *Mycobacterium leprae*. Microbiology and Immunology. 1983;**27**:409-414

[89] Tønjum T, Welty DB, Jantzen E, Small PL. Differentiation of *Mycobacterium ulcerans, M. marinum* and *M. haemophilum*: Mapping of their relationships to *M. tuberculosis* by fatty acid profile analysis, DNA-DNA hybridization and 16S rRNA gene sequence analysis. Journal of Clinical Microbiology. 1998;**36**:918-925

[90] Hershkovitz I, Donoghue HD, Minnikin DE, Besra GS, Lee OY-C, Gernaey AM, Galili E, Eshed V, Greenblatt CL, Lemma E, Kahila Bar-Gal G, Spigelman M. Detection and molecular characterization of 9000-year-old *Mycobacterium tuberculosis* from a Neolithic settlement in the Eastern Mediterranean. PLoS One. 2008;**3**:e3426

[91] Redman JE, Shaw MJ, Mallet AL, Santos AL, Roberts CA, Gernaey AM, Minnikin DE. Mycocerosic acid biomarkers for the diagnosis of tuberculosis in the Coimbra skeletal collection. Tuberculosis. 2009;**89**:267-277. DOI: 10.1016/j.tube.2009.04.001

[92] Lee OY-C, Wu HHT, Donoghue HD, Spigelman M, Greenblatt CL, Bull ID, Rothschild BM, Martin LD, Minnikin DE, Besra GS. *Mycobacterium tuberculosis* complex lipid virulence factors preserved in the 17,000-year-old skeleton of an extinct bison, *Bison antiquus*. PLoS One. 2012b;**7**:e41923. DOI: 10.1371/journal.pone.0041923

[93] Blau S, Yagodin V. Osteoarchaeological evidence for leprosy from Western Central Asia. American Journal of Physical Anthropology. 2005;**126**:150-158. DOI: 10.1126/science.aah3783

[94] Blau S, Yagodin VAMS. AMS radiocarbon dates of kurgans located on the Ust'-Yurt plateau, Uzbekistan. Radiocarbon. 2005;**47**:235-241

[95] Hansen GHA. Undersogelser angaende spedalskhedens aasager. Norsk Magazin for Laegervidens-kaben. 1874;**4**(Suppl):1-88

[96] Hohmann N, Voss-Böhme A. The epidemiological consequences of leprosy-tuberculosis co-infection. Mathematical Biosciences. 2013;**241**:225-237. DOI: 10.1016/j.mbs.2012.11.008

[97] Nuorala E, Donoghue HD, Spigelman M, Götherström A, Hårding B, Grundberg L, Alexandersen V, Leden I, Lidèn K. Diet and Disease in Björned, a Viking-Early Medieval Site in Northern Sweden. Ancient DNA Analyses of the Bacterial Diseases Tuberculosis and Leprosy. Theses and Papers in Scientific Archaeology. Vol. 6. Stockholm: Archaeological Research Laboratory, Stockholm University; 2004

[98] Cole ST, Eiglmeler K, Parkhill J, James KD, Thomson NR, Wheeler PR, Honoré N, Garnier T, Churcher C, Harris D, Mungall K, Basham D, Brown D, Chillingworth T, Connor R, Davies RM, Devlin K, Duthoy S, Feltwell T, Fraser A, Hamlin N, Holroyd S, Hornsby T, Jagels K, Lacroix C, Maclean J, Moule S, Murphy L, Oliver K, Quail MA, Rajandream M-A, Rutherford KM, Rutter S, Seeger K, Simon S, Simmonds M, Skelton J, Squares R, Squares S, Stevens K, Taylor K, Whitehead S, Woodward JR, Barrell BG. Massive gene decay in the leprosy bacillus. Nature. 2001;**409**:1007-1011

[99] Cole ST, Supply P, Honoré N. Repetitive sequences in *Mycobacterium leprae* and their impact on genome plasticity. Leprosy Review. 2001;**72**:449-461

[100] Gómez-Valero L, Rocha EPC, Latorre A, Silva FJ. Reconstructing the ancestor of *Mycobacterium leprae*: The dynamics of gene loss and genome reduction. Genome Research. 2007;**17**:1178-1185

[101] Robbins G, Tripathy VM, Misra VN, Mohanty RK, Shinde VS, Gray KM, Schug MD. Ancient skeletal evidence for leprosy in India (2000 B.C.). PLoS One. 2009;**4**:e5669. DOI: 10.1371/journal.pone.0005669

[102] Mignard S, Flandrois J-P. A seven-gene, multilocus, genus-wide approach to the phylogeny of mycobacteria using supertrees. International Journal of Systematic and Evolutionary Microbiology. 2008;**58**:1432-1441. DOI: 10.1099/ijs.0.65658-0

[103] Prasanna AN, Mehra S. Comparative phylogenomics of pathogenic and non-pathogenic *Mycobacterium*. PLoS One. 2013;**8**:e71248

[104] Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG. Fast, Scalable generation of high-quality protein multiple sequence alignments using Clustal omega. Molecular Systems Biology. 2011;**7**:539

[105] Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution. 2016;**33**:1870-1874

[106] Sompolinsky D, Lagziel A, Naveh D, Yankilevitz T. *Mycobacterium haemophilum* sp. nov., a new pathogen of humans. International Journal of Systematic Bacteriology. 1978;**28**: 67-75

[107] Sompolinsky D, Lagziel A, Rosenberg I. Further studies of a new pathogenic *Mycobacterium* (*M. haemophilum* sp. nov.). Canadian Journal of Microbiology. 1979;**25**:217-226

[108] Kelley CF, Armstrong WS, Eaton ME. Disseminated *Mycobacterium haemophilum* infection. The Lancet Infectious Diseases. 2011;**11**:571-578

[109] Copeland NK, Arora NS, Ferguson TM. *Mycobacterium haemophilum* masquerading as leprosy in a renal transplant patient. Case Reports in Dermatological Medicine. 2013; Article ID 793127. DOI: 10.1155/2013/793127

[110] Ishii K, Ishii N, Nakanaga K, Nakano K, Saito I, Asahina A. *Mycobacterium haemophilum* infection with prominent facial manifestation mimicking leprosy. The Journal of Dermatology. 2015;**42**:992-995. DOI: 10.1111/1346-8138.12948

[111] SoRelle JA, Beal SG, Scollard DM, Gander RM, Cohen J, Nuara A, Nations S, Cavuoti D. *Mycobacterium leprae* and *Mycobacterium haemophilum* co-infection in an iatrogenically immunosuppressed patient. Diagnostic Microbiology and Infectious Disease. 2014;**78**:494-496. DOI: 10.1016/j.diagmicrobio.2013.09.003

[112] Donnelly K, Waltzek TB, Wellehan JFX Jr, Stacy NI, Chadam M, Stacy BA. *Mycobacterium haemophilum* infection in a juvenile leatherback sea turtle (*Dermochelys cori*acea). Journal of Veterinary Diagnostic Investigation. 2016;**28**:718-721

[113] Becherer P, Hopfer RL. Infection with *Mycobacterium haemophilum*. Clinical Infectious Diseases. 1992;**14**:793

[114] Sharma R, Lahiri R, Scollard DM, Pena M, Williams DL, Adams LB, Figarola J, Truman RW. The armadillo: A model for the neuropathy of leprosy and potentially other neurodegenerative diseases. Disease Models & Mechanisms. 2013;**6**:19-24. DOI: 10.1242/dmm.010215

[115] Sharma R, Singh P, Loughry WJ, Lockhart JM, Inman WB, Duthie MS, Pena MY, Marcos LA, Scollard DM, Cole ST, Truman RW. Zoonotic leprosy in the southeastern United States. Emerging Infectious Diseases. 2015;**21**:2127-2134. DOI: 10.3201/eid2112.150501

[116] Pin D, Guérin-Faublée V, Garreau V, Breysse F, Dumitrescu O, Flandrois J-P, Lina G. *Mycobacterium* species related to *M. leprae* and "*M. lepromatosis*" from cows with bovine nodular thelitis. Emerging Infectious Diseases 2014;**20**:2111-2114. DOI: http://dx.doi.org/10.3201/eid2012.140184

[117] Chartier C, Albaric O, Cesbron N, Despres J, Hoogveld C, Michalet L, Boschiroli M-L. Tuberculoid nodular thelitis in a dairy goat flock. Veterinary Journal. 2016;**209**:199-200. DOI: 10.1016/j.tvjl.2015.12.004

[118] Malik R, O'Brien CR. Leprosy–we've much left to learn, but are looking to squirrels, cows and cats for insights. Journal of Feline Medicine and Surgery. 2017;**19**:977-978. DOI: 10.1177/1098612X17723248

[119] O'Brien C, Malik R. History and mysteries of leprosy. Journal of Feline Medicine and Surgery. 2017;**19**:496-497. DOI: 10.1177/1098612X17706460

[120] O'Brien C, Malik R, Globan M, Reppas G, McCowan C, Fyfe JA. Feline leprosy due to *Candidatus* 'Mycobacterium tarwinense'. Further clinical and molecular characterization of 15 previously reported cases and an additional 27 cases. Journal of Feline Medicine and Surgery. 2017;**19**:498-512. DOI: 10.1177/1098612X17706467

[121] O'Brien C, Malik R, Globan M, Reppas G, McCowan C, Fyfe JA. Feline leprosy due to *Candidatus* 'Mycobacterium lepraefelis': Further clinical and molecular characterisation of eight previously reported cases and an additional 30 cases. Journal of Feline Medicine and Surgery. 2017;**19**:919-932. DOI: 10.1177/1098612X17706470

[122] Hughes MS, Ball NW, Beck L-A, De Lisle GW, Skuce RA, Neill SD. Determination of the etiology of presumptive feline leprosy by 16S rRNA gene analysis. Journal of Clinical Microbiology. 1997;**35**:2464-2471

[123] Malik R, Hughes MS, James G, Martin P, Wigney DI, Canfield PJ, Chen SCA, Mitchell DH, Love DN. Feline leprosy: Two different clinical syndromes. Journal of Feline Medicine and Surgery. 2002;**4**:43-59. DOI: 10.1053/jfms.2001.0151

[124] Hughes MS, James G, Taylor MJ, McCarroll J, Neill SD, Chen SCA, Mitchell DH, Love DN, Malik R. PCR studies of feline leprosy cases. Journal of Feline Medicine and Surgery. 2004;**6**:235-243. DOI: 10.1016/j.jfms.2003.09.003

[125] Appleyard GD, Clark EG. Histologic and genotypic characterization of a novel *Mycobacterium* species found in three cats. Journal of Clinical Microbiology. 2002;**40**:2425-2430. DOI: 10.1128/JCM.40.7.2425-2430.2002

[126] Fyfe JA, McCowan C, O'Brien CR, Globan M, Birch C, Revill P, Barrs VRD, Wayne J, Hughes MS, Holloway S, Malik R. Molecular characterization of a novel fastidious *Mycobacterium* causing lepromatous lesions of the skin, subcutis, cornea, and conjunctiva of cats living in Victoria, Australia. Journal of Clinical Microbiology. 2008;**46**:618-626. DOI: 10.1128/JCM.01186-07

[127] Han XY. Detection of the leprosy agent "*Mycobacterium lepromatosis*" in South America and Europe. The American Journal of Tropical Medicine and Hygiene. 2017;**96**:260

[128] Fedrizzi T, Meehan CJ, Grottola A, Giacobazzi E, Serpini GF, Tagliazucchi S, Fabio A, Bettua C, Bertorelli R, De Sanctis V, Rumpianesi F, Pecorari M, Jousson O, Tortoli E, Segata N. Genomic characterization of nontuberculous mycobacteria. Scientific Reports. 2017;**7**:45258. DOI: 10.1038/srep45258