Molecular epidemiology of leprosy: an update

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Abstract

Molecular epidemiology investigations are notoriously challenging in the leprosy field mainly because the inherent characteristics of the disease as well as its yet uncultivated causative agents, *Mycobacterium leprae* and *M. lepromatosis*. Despite significant developments in understanding the biology of leprosy bacilli through genomic approaches, the exact mechanisms of transmission is still unclear and the factors underlying pathological variation of the disease in different patients remain as major gaps in our knowledge about leprosy. . Despite these difficulties, the last two decades have seen the development of genotyping procedures based on PCR-sequencing of target loci as well as by the genome-wide analysis of an increasing number of geographically diverse isolates of leprosy bacilli. This has provided a foundation for molecular epidemiology studies that are bringing a better understanding of strain evolution associated with ancient human migrations, and phylogeographical insights about the spread of disease globally. This review discusses the advantages and drawbacks of the main tools available for molecular epidemiological investigations of leprosy and summarizes various methods ranging from PCR-based genotyping to genome-typing techniques. We also describe their main applications in analyzing the short-range and long-range transmission of the disease. Finally, we summarise the current gaps and challenges that remain in the field of molecular epidemiology of leprosy.

Author contribution

PNS was invited to write the review and gathered the team.

All authors contributed to the manuscript.

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1. Introduction

Leprosy is a neglected tropical disease caused by infection with *Mycobacterium leprae* or *M. lepromatosis*. Following a massive decades-long worldwide campaign of treatment with multi-drug therapy (MDT) (Smith et al., 2017), this chronic disease was declared to be eliminated worldwide (less than one per 10,000 inhabitants) by the World Health Organization (WHO) in 2000. Subsequently, several countries have achieved that elimination target individually (Richardus et al., 2016; Schaub et al., 2020; World Health Organization, 2008). Nevertheless, new cases continue to evolve around the world and in 2018, nearly 250,000 new leprosy cases were reported from 131 countries, with 95% of those detected mainly in India, Brazil, Indonesia and 20 other global priority countries (WHO, 2019). Pockets of high endemicity remain even within some countries which have achieved the 'elimination' goal, as large numbers of hidden cases are often discovered following local intensive case detection campaigns (Blok et al., 2016; de Sousa et al., 2020; Kumar et al., 2013; Rao and Suneetha, 2018; Salgado et al., 2018, 2016; Smith et al., 2015). Additionally, 7.6% (n=16,013) of the new leprosy cases detected globally still occurs among children (with 96% of all cases in the 23 global priority countries), suggesting there is continuing active transmission of the disease within some communities.

Untreated, leprosy symptoms progress to irreversible physical disabilities such as blindness and limb deformities. Current leprosy control strategies rely on early diagnosis and prompt treatment to minimize the progressive morbidity of leprosy and hopefully interrupt transmission from clinically active cases (Smith et al., 2014). However, this strategy is still not optimally implemented and 5.4% of the new cases were diagnosed with advanced leprosy disabilities in 2018 for which 90% were found in the global priority countries (WHO, 2019). Early leprosy care is impaired by a combination of factors, including: a) stigma about leprosy, which remains strong in most communities, and inhibits many individuals from seeking healthcare (Price, 2017); b) the absence of a rapid and sensitive diagnostic test for all clinical forms and early stages of leprosy (Steinmann et al., 2017); c) an incomplete understanding of leprosy transmission and awareness of the disease in many communities (Mensah-Awere et al., 2015), d) the lack of tools to monitor drug efficacy (Cambau et al., 2018) and, e) poor healthcare system in some endemic countries (WHO, 2015).

Although the exact mode of transmission of the leprosy bacilli has not been elucidated, close contact with an infected individual in the same household or community is commonly

recognized as a high-risk factor for infection (Goulart et al., 2008). Leprosy is considered to be lowly contagious and it does not spread easily in communities. The disease manifests over a broad clinical and histopathological spectrum ranging from tuberculoid to lepromatous depending on the individual host response. The tuberculoid (paucibacillary spectrum) is characterized by active cell mediated immunity with well-defined granulomas containing relatively few bacilli, while the lepromatous spectrum (multibacillary spectrum) shows increased humoral immune responses with poorly organized or diffuse granulomas containing large numbers of bacilli. Owing to the large numbers of bacilli they may shed, multibacillary cases are typically thought to have a greater role in transmitting the disease, but paucibacillary cases also can transmit the infection. Leprosy bacilli are found on the nasal mucosa and on the skin of active multibacillary cases, as well as up to 5% of asymptomatic individuals in endemic areas. Both anatomic sites have long been thought to be the primary routes of entry for the bacilli into the human host (Bratschi et al., 2015; Kumar et al., 2016). Adding to the complex picture, with an incubation of three to five years to manifest clinical illness, asymptomatic pre-clinical cases may shed the bacilli to others as their disease progresses (Araujo et al., 2016; Frade and Foss, 2016) In addition, some non-human animal also have been implicated in transmitting leprosy while possible environmental reservoirs have been identified in the last decade.

To establish leprosy transmission patterns numerous epidemiological investigations have focused on general clinical or social factors such as age (Vieira et al., 2018), gender (Sarkar and Pradhan, 2016), disease type (Nobre et al., 2017), standard of living (Kerr-Pontes et al., 2004; Lockwood, 2004; Serrano-Coll et al., 2019), diet (Oktaria et al., 2018), spatial distribution (Wangara et al., 2019), chronology (Nazario et al., 2017) as well as familial genetics (Cambri and Mira, 2018), and other factors. Although a review of all these aspects of leprosy is beyond the scope of this article, comprehensive updates on many of these and other aspects of leprosy is available via the online textbook "International Textbook of Leprosy". (https://internationaltextbookofleprosy.org/)

In parallel, molecular epidemiology of infectious diseases has grown rapidly in the past decades with the advances in DNA-based molecular typing techniques such as genotyping methods and next-generation sequencing (Wang et al., 2015). These techniques have contributed to a better understanding of the etiology, transmission and spatial distribution of a variety of infectious

diseases and give insight into the genetic variability and evolution of their causative agents (Wang et al., 2015). However, these methods are still costly, technically complex and difficult to apply to leprosy agents.

Leprosy is caused most commonly by *Mycobacterium leprae* and less frequently by *M. lepromatosis.* Both are obligate intracellular pathogens and among the slowest growing bacteria known. In animal models their doubling times range from 10-12 days (Sharma et al., 2019; Truman and Krahenbuhl, 2001). Neither organism has been successfully cultured on artificial media in the laboratory, which significantly impairs our ability to recover sufficient quantities of high quality genetic material for molecular studies (Britton and Lockwood, 2004; Pattyn, 1973). Samples for molecular investigation are usually restricted to only minute volumes of sample where bacilli are dabbed directly from patient skin lesions, including slit skin smear (SSS); nasal swabs (NS) or biopsies of leprosy lesions, a procedure which is both technically difficult and invasive. All samples yield variable amounts of bacteria depending on the bacillary load correlated with the form of disease they manifest. Samples from tuberculoid (TT) or paucibacillary patients contain a very low numbers of bacteria and are considered the most challenging (Barbieri et al., 2019). Most of our knowledge to date has come from studies on samples taken from patients in the multibacillary bacillary spectrum, simply because of the relatively higher abundance of recoverable bacilli in their tissues.

Despite these difficulties, the last two decades have seen the development of a wide variety of genotyping procedures and the establishment of a foundation for molecular epidemiological studies of leprosy (Salipante and Hall, 2011). In 2011, two publications reviewed precisely the methods available for strain typing, the challenges around their development for the leprosy field and the application of these markers in transmission studies (Salipante and Hall, 2011; Singh and Cole, 2011). Now about a decade later, we aim to capture the state of molecular epidemiology in leprosy by describing the evolution of these procedures, the development of new methods and approaches, as well as their main applications and outcomes and summarize the major gaps that need to be overcome in the future.

2. Rise and evolution of molecular epidemiological markers for leprosy

2.1. The genotyping systems

Slow growing pathogenic mycobacteria present very low variability on the DNA level and are considered to be genetically monomorphic or clonal (Comas et al., 2009). While the genetic substitution rate of *M. tuberculosis,* the causative is estimated to be about 10^{-7} to 10^{-8} substitutions per site in the genome per year (Duchêne et al., 2016), that of *M. leprae* is even lower at $7.8x10^{-9}$ substitutions per site in the genome per year making it among some of the most highly conserved of all bacteria (Benjak et al., 2018). As consequence, molecular studies on *M. leprae* often rely on genomic elements sometimes considered too variable for use in other bacterial systems. Molecular typing markers for *M. leprae* are mainly based on two groups of genetic markers: short nucleotide sequences located adjacent to each other on the chromosome that vary in copy number between different bacterial strains known as variable number of tandem repeats (VNTR) and single nucleotide polymorphisms (SNP), insertions and deletions (InDels).

In the pre-genomic era, two VNTR loci were described that showed variability between strains from different countries (*rpoT* hexamer) or locally (GAA)21 (Matsuoka et al., 2000; Shin et al., 2000). However, their resolution was limited (Salipante and Hall, 2011). Later, the availability of the complete *M. leprae* genome (Cole et al., 2001) allowed identification of an additional 33 microsatellites (repeat units of less than 6 bp) and 11 minisatellites (repeat units of 6 to 100 bp) loci (Groathouse et al., 2004) from which a panel of 16 was eventually characterized and validated as discriminatory using clinical isolates (>500) from six countries (Cardona-Castro et al., 2009; Fontes et al., 2009; Gillis et al., 2009; Kimura et al., 2009, p.; Matsuoka et al., 2009; Sakamuri et al., 2009b, 2009a; Shinde et al., 2009; Srisungnam et al., 2009; Xing et al., 2009). Gillis and colleagues showed that the loci (AT)15 and (TA)18 were not reproducible and that (GAA)21 was not stable during *in vivo* passage in mice and could not be used as reliable marker (Gillis et al., 2009). Similarly, they found technical issues when amplifying 18-8 but otherwise found reliable amplification at other loci with as few as 10-cells. In examining a large volume of sequence data from six countries, Salipante and Hall confirmed these observations and suggested that (AT)15 and (TA)18 loci should be removed from future analysis (Hall and Salipante, 2010) or at least be locally validated for individual populations (Fontes et al., 2009).

Comparing the genomes of four *M. leprae* strains from India, Brazil, Thailand and the United States revealed 84 informative markers capable of discriminating 4 SNP-types based on three distinct loci and 16 *M. leprae* SNP-subtypes 1A-D, 2E-H, 3I-M and 4N-P (Monot et al., 2009, 2005). Mapping these polymorphisms in more than 400 strains arsing from 28 different countries showed that the distribution of SNP-subtypes was correlated with the geographical origin of the patients, and suggested that SNP-typing could be a robust tool for future phylogeographic and evolutionary studies (Monot et al., 2009).

Inherent characteristics of both SNP- and VNTR-typing were already suggestive of the range of their future applications. Indeed, VNTR polymorphisms arise from bacterial replication slippage and are therefore less stable. However, using a select set of 14 validated loci (Gillis et al., 2009), VNTR-typing could be used effectively discriminate short-range transmission networks on a village- or family-scale (Singh and Cole, 2011). As SNPs are far more stable and less prone to variation in short time intervals, SNP-typing retains its best application in long-range transmission such as country-wide or global dissemination, or in historical time periods (Singh and Cole, 2011). A combined approach of the 14 VNTR loci and 16 SNP sub-type was proposed to maximize the power of molecular epidemiology and effectively used for providing a strong evidence about the zoonotic link of armadillo with human leprosy in the southern United States (Truman et al., 2011). This approach of combining SNPs and VNTRs has been recommended in subsequent publications (Salipante and Hall, 2011; Singh and Cole, 2011).

In parallel, whole-genome sequencing (WGS) was also acknowledged as a powerful tool for genotyping since it allows a deeper resolution into the overall genetic variability of each isolate and provides robust data for population-based analyses (Salipante and Hall, 2011; Singh and Cole, 2011). In 2009, only four *M. leprae* whole-genome sequences were available, but even this limited amount of data was usefulfor developing a robust typing system and yielded unprecedented information on strain diversity and evolution [**section 3**]. This observation was the first hint that a whole-genome approach could rapidly overcome the challenge of standard typing systems for *M. leprae*. However, massive technical challenges, cost, labor intensity and time-consuming downstream analysis limited its application (Salipante and Hall, 2011; Singh and Cole, 2011).

2.2. The combined SNP and VNTR-typing approach

Genotyping studies on *M. leprae* have rapidly expanded since 2011 with at least 53 different research articles published on the subject. These studies involved a variety of genotyping methods, including 14 studies (26%) that used next-generation sequencing (Supplementary **Table**). Most investigations originated from India (n=12), Brazil (n=11), China (n=6) and Colombia (n=3). The combined approach of VNTR and SNP typing was conducted in 13 studies (24%) including three in which WGS was also performed. However, the validated dataset of SNP subtyping (Monot et al., 2009) and VNTR- typing (Gillis et al., 2009) was available only in seven studies (13%) (Avanzi et al., 2016a; Dai et al., 2019; Kuruwa et al., 2012; Sharma et al., 2015; Stefani et al., 2017; Truman et al., 2011; Weng et al., 2013b) and more recently this number increases to nine with two studies in Brazil using VNTR and SNP-typing (Fontes et al., 2017; Rosa et al., 2019).

Several reasons might explain the poor utilization of the full combination of VNTR and SNP-subtyping in the molecular epidemiological studies from the last decade. First, despite the improvements facilitated by multiplex PCR (Shinde et al., 2009), PCR amplification of all VNTR and SNP positions requires a fair amount of starting material and the number of loci required to be amplified depends largely on the diversity of genotypes present in a given locale. For example, in India, SNP subtyping requires fewer amplifications since only SNP types 1 and 2 are presented, while in Brazil three different SNP types (1, 3 and 4) are commonly found (**Figure 1**).

Additionally, different studies showed that despite their robust diversity globally, a combination of VNTR and SNPs might be more informative than others in certain geographic locations, such as India (Lavania et al., 2015) or Colombia (Cardona-Castro et al., 2013). Similarly, Cardona-Castro and colleagues identified three different genotypes (Cardona-Castro et al., 2013) circulating in Colombia based on the combination of one informative site (a polymorphism in *gyrA* at genomic coordinate 7614 which is specific to the 3I genotype) (Truman et al., 2011) and only three VNTR loci [**part 3.1.5**]. This polymorphism, called also C497T, had independently been described in Brazil by da Silva Rocha et al. and discriminated SNP-type 3 genotypes from others (da Silva Rocha et al., 2011). Although VNTRs are considered less reliable for phylogeny, all mentioned studies showed a certain level of association between VNTRs and SNP-type. Indeed, different groups demonstrated that two allelic patterns of VNTR 27-5 and 12-5 were associated with SNP-type 3 in different states in

Brazil and in Colombia (Cardona-Castro et al., 2013; Fontes et al., 2017, 2009). However, owing to the differences in prevalence of *M. leprae* genotypes in different locations, the application of these methods can have limited utility and must be validated for each geographic area.

Similarly, new informative polymorphisms not previously used in the SNP typing systems were identified in different countries, such as variants in the 16S sequence of strains from China (Yuan et al., 2015) and the gene *folP1* coding for the dihydropteroate synthase in Indonesia (Maladan et al., 2019). However, the prevalence of these markers in *M. leprae* strains from other countries has not been assessed and their use remains limited.

The final reason is the absence of guidelines or consensus techniques to combine VNTR and SNP data. Currently, data are mostly used separately, leading to the definition of 16 SNP-subtypes and up to 417 genotypes from 465 isolates of *M. leprae* using VNTRs data alone (Hall and Salipante, 2010), the latter now increased to a local database from over 1.500 isolates from eight different countries (P. Suffys, personal communication). Several studies have suggested to increase the resolution in each SNP branching by including the higher variability with a panel of VNTRs, thereby largely overcoming the limitations of the individual systems and complementing the definition of a genotype by SNP subtype, followed by internal cluster identification using VNTR patterns (Singh and Cole, 2011; Truman et al., 2011). This approach has been used to differentiate strains with very low genetic variability where the resolution of WGS was not sufficient (Avanzi et al., 2016a; Rosa et al., 2019; Stefani et al., 2017; Truman et al., 2011).

2.3. Whole-genome sequencing of *Mycobacterium leprae* strains

2.3.1. Methodology

Efficient WGS of uncultivable bacteria directly from clinical samples is challenging owing to vast abundance of the host genomic DNA contaminating the reaction. For *M. leprae*, whole-genome sequences can be obtained from clinical strains cultured in *in vivo* models, or following metagenomic sequencing on DNA retrieved from samples with sufficient genetic material without prior enrichment (Cole et al., 2001; Guan et al., 2020; Monot et al., 2009; Schuenemann et al., 2013a; Truman et al., 2011). Nevertheless, such an approach is very time-consuming

and often ineffective when dealing with samples containing relatively low numbers of bacilli such as NS (nasal swabs), SSS (slit skin smears), fixed skin samples, blood, human remains or samples from paucibacillary patients. In recent years, a couple of methods have been developed to overcome this challenge by either capturing *M. leprae* DNA using hybridization-based capture on an array or in-solution with biotinylated RNA probes (Honap et al., 2018; Schuenemann et al., 2013a). Or in parallel, with mechanical enrichment also removing host DNA during DNA extraction (Avanzi et al., 2016b). The host DNA depletion technique can only be applied on skin biopsies and remains largely ineffective for the paucibacillary samples (Benjak et al., 2018). However, thanks to these different approaches, the number of whole genomic sequences of leprosy bacilli now available has increased from only four in 2009 to more than 250 in 2020 (**Figure 2**) (Tió-Coma et al., 2020a).

2.3.2. Improvement of genotyping based on genome-typing and limitations

In the last 10 years, the increased number of genomes available from around the world has helped to refine basic genotyping systems (**Table**) aided by the identification of new genotypes (Avanzi et al., 2020a; Benjak et al., 2018; Stefani et al., 2017; Tió-Coma et al., 2020a), the misclassification of others (Tió-Coma et al., 2020a) and the deeper resolution inside genotypes (Benjak et al., 2018; Sharma et al., 2015; Singh et al., 2014; Truman et al., 2011). In a recent study performed in Madagascar and the Comoros, WGS was used to decipher specific regionally associated SNPs of the main strain circulating on the island, and used them for genotyping of DNA samples that otherwise were not suitable for WGS (Avanzi et al., 2020a). This approach allowed the characterization of a unique genotype in the region and provided a means to assess its distribution.

This approach has two mains drawbacks: (i) WGS must be performed to obtain the genetic background of the strains circulating in a given area and, (ii) additionally, in countries where several genotypes are circulating (such as India and Brazil), several rounds of PCR-sequencing per sample might be required with this kind of approach. This could become time-consuming and costly especially if combined with molecular drug susceptibility investigations. While the first point is inevitable and might require a high number of strains sequenced in case of large countries, the second point might be optimized using targeted sequencing.

Targeted sequencing can be performed in two main approaches: amplicon or capture-based approach. Amplicon based-enrichment allows the amplification of regions of interest prior to library preparation while captured-based enrichment is used to fish these specific regions after library preparation. Amplicon-based enrichment is usually less costly, especially if multiplex PCR can be applied. It also requires much less starting material than capture therefore allowing direct application from clinical specimens. Additionally, targeted sequencing can be used on different platforms, including the Illumina machines and the more user-friendly and bench devices such as the MinION (Dolinger et al., 2016). Targeted sequencing was successfully used in the tuberculosis field for characterization to the lineage level and of molecular drug susceptibility of *M. tuberculosis* (Colman et al., 2019; Makhado et al., 2018). A similar test is under development for *M. leprae* spanning a panel of markers for genotyping and drug susceptibility testing applicable directly in clinical samples (Philip Supply, Philip Suffys and Bouke de Jong, personal communication)

3. Application of molecular epidemiology since 2009

3.1. Dynamics of *Mycobacterium leprae* transmission in leprosy-endemic countries

In the last ten years, molecular investigations on strain distribution were conducted at the national level mainly in India, Brazil and China. India and Brazil reported the highest number of new leprosy cases in 2018. Although India achieved the WHO elimination goal at the national level as early as 2005, pockets of high-endemicity remain and the new case detection rate in India has remained greater than 127,000 annually over the last decade, representing some 60% of the global total cases (Rao and Suneetha, 2018). China also attained the elimination target at a national level in the 1980's but they too continue to have pockets of high endemicity, especially in the South, where incidences of leprosy are higher than the national average (Sun et al., 2019; WHO, 2019). Likewsie, Brazil too differing rates of transmission with annual prevalence rates ranging from 0.14 to 15.52/10,000 inhabitants, respectively in the states of Rio Grande do Sul (South) and Mato Grosso (in the Amazonian region) in 2018 (Ministério da Saúde, 2019). It is thus important to understand the dynamics of *M. leprae* transmission in such foci of active transmission so that the new case detection rate can be further reduced with effective control strategies.

3.1.1. Brazil

In 2009, VNTR- and SNP-typing were performed in the state of Rio de Janeiro and São Paulo, as an initial study on the bacterial population structure of *M. leprae* in some endemic regions of the country and demonstrating highly variable VNTR patterns among unrelated strains and a predominance of SNP type 3 in both states (Fontes et al., 2009). A link between the VNTR profile 27-5 and 12-5 was also observed with allele 4/5 for SNP type 3 and 5/4 for SNP type 4 (Fontes et al., 2009). Later, the high prevalence of SNP type 3 in Rio de Janeiro and the predominance of SNP type 4 in Northeast states of the country also was confirmed, suggesting a differential introduction of type 4 strains to northeast Brazil likely through imported laborers and the slave trade (Fontes et al., 2012). Additionally, that study suggested a partial region-associated clustering of VNTR patterns, but epidemiologic data were lacking to support a possible transmission event.

A later molecular investigation was carried out in the city of Fortaleza, in the state of Ceará, an endemic region for leprosy with a considerable number of highly endemic municipalities (Fontes et al. 2017). VNTR typing could be performed from 16% of the patients diagnosed during the study period with clustering level being around 60% when excluding highly discriminatory STRs but their nature and number should be evaluated locally. Besides being indicative for high level of recent transmission, clustering was also associated with a late notification of the disease and with grade 2 disability and with some neighborhoods of the city.

More recent and detailed SNP-typing studies reported a similar trend with the genotype 4 prevalent in the North-East while genotype 3 seems more common in the South-eastern states (Benjak et al., 2018). Several subtypes are circulating is Brazil, probably reflecting the historical successive waves of colonization and exchanges with Europe and West Africa. In Rio de Janeiro and Sao Paulo, *M. leprae* harbored mainly the genotype 3I-2 and only a few strains from Sao Paulo showed the genotype 3I-1, which is relatively closer to the medieval European strains **(Figure 1)** (Benjak et al., 2018). In North and North-eastern states, the 4N genotype is mainly reported with some genotype 4P (Benjak et al., 2018). The genotype 1D is sporadically identified in these states. Finally, a new genotype, named 4N/O **(Table)**, was identified in a relapse case from Ceará state (Stefani et al., 2017). So far, this genotype was observed only in one patient from Niger and in non-human primates from West Africa (Benjak et al., 2018; Honap et al., 2018).

3.1.2. India and neighboring countries

Molecular epidemiology of *M. leprae* strains in India has used the VNTR and SNP-subtyping in several regions. Similar to Brazil, different combinations of loci 27-5 and 12-5 were observed in different regions with alleles of the two loci combined as 4/4, 4/5, 5/4 and 5/5 while (Kuruwa et al., 2012; Lavania et al., 2011; Shinde et al., 2009).

The 1D and 1C SNP-subtypes are the primary genotypes circulating in India, but they show no specific regionally associated trends (**Figure 1**). Recently, greater diversity has been recognized in the genotype 1D when using WGS showing that 1D-2 strains are mainly present in East Asia while the 1D-1 clade is composed of strains from West Africa and South America. Added to this is the discovery of a new genotype (1D-Malagasy) circulating in Madagascar and the classification of the 1C as 1D-genotype inside the 1D-2 (Avanzi et al., 2020a; Tió-Coma et al., 2020a) (**Table**).

In parallel, the genotype 2G, 1A, 2E and 1B are observed, though they constitute a very small proportion in most of the places in India with the genotype 1B identified in only three patients from Maharashtra (**Figure 1**). The genotype 2E, hypothesized to be the ancestor of the genotype 1, mostly reported from East Africa and Yemen (Avanzi et al., 2020a; Benjak et al., 2018), is observed in the Northern regions. Interestingly, the genetic diversity of *M. leprae* strains in Rajasthan, Uttar Pradesh and West Bengal are similar to the one circulating from neighboring countries such as in Pakistan (Benjak et al., 2018), Nepal (Avanzi et al., 2020a; Monot et al., 2009) and Bangladesh (Tió-Coma et al., 2020a), respectively, but completely different when compared to China where the 3K is widely distributed **(Figure 1)**. The 3K-genotype was never observed among 479 strains genotyped from India while the genotype 2 was reported in one patient from the western part of China, in the Xinjiang region (**Figure 1**). This difference could be associated with the presence of the natural barrier, the Himalayas, between the different countries decreasing the contact between autochthonous populations. In Bangladesh, only one study was performed and reported the genotype 1D, 1A and a new genotype called 1B-Bangladesh (Tió-Coma et al., 2020a). However, this study was conducted in only one region (North) and therefore probably does not represent the overall *M. leprae* diversity in the country.

Deep sequence genotyping or WGS of strains at regional and country level is still in its infancy but hold promise to identify possible diversity, retrace the origin of the strains circulating in

this part of the world hosting the oldest skeleton containing traces of leprosy scars (Robbins et al., 2009) and, coupled with *in vivo* characterization, could reveal associations between genotype and level of pathogenicity, if any.

3.1.3. China

Prior to 2009, molecular investigations revealed little genetic diversity of *M. leprae* in China (genotype 3K only) (Monot et al., 2009). Later, two investigations led by Weng and colleagues showed more variability when analyzing also the VNTR profiles in four provinces and villages (Weng et al., 2013a, 2011). In Yunnan, a province reporting the highest leprosy prevalence in 2018 (Sun et al., 2019), previous investigations showed different VNTR allele frequencies when comparing the northern, eastern and southern cities (Weng et al., 2011). In this eight-year study, allele frequencies were also not found to significantly differ between genders or clinical presentations. Later, extending their investigation in 17 provinces, they confirmed the wide representation of the genotype 3K but also identified the genotype 1D in three coastal provinces and the genotype 2 in one strain from the West province of Xinjiang (**Figure 1**) (Weng et al., 2013a; Yuan et al., 2015).

WGS of genotype 3K recently revealed greater diversity in *M. leprae* strains from East Asia and Pacific Island with the identification of the genotype 3K-1 in Japan, Pacific Islands and the Philippines (Benjak et al., 2018; Guan et al., 2020). In China, there is currently only one genome sequenced and the strain belongs to the ancestral genotype 3K-0, such as others from Japan, Korea, New Caledonia and the Pacific Island (Benjak et al., 2018; Schuenemann et al., 2013a). The presence of the genotype 3K-1 in China is not yet documented.

3.1.4. Other countries

Molecular investigations have been conducted somewhat non-systematically in some other countries with varying endemicity rates (WHO, 2019). In Indonesia, a country with high endemicity that typically reports the third highest number of new cases annually (WHO, 2019), only two studies on *M. leprae* strain typing have been published and both appeared in 2019. One molecular drug susceptibility study reported a possible new informative position in *folP1*

gene, specific to the strains circulating in Indonesia (Maladan et al., 2019) while the other reported the distribution of different clusters in two regions on the basis of four VNTR loci (Prakoeswa et al., 2019). With more than 17,000 new cases reported in 2018 (WHO, 2019), molecular investigations are needed to better understand the dynamics of leprosy transmission in this country.

In Colombia, 400-500 new cases are reported every year but leprosy prevalence remains above the goal of 1 case per 10,000 population in several regions (Cardona-Castro, 2018). Molecular typing of the *M. leprae* strains has been investigated in all regions (Cardona-Castro et al., 2015). Three genotypes representing a combination of alleles from two VNTR loci (12-5 (4/5) and 21-3 (6/5/4)) and one missense mutation in *gyrA* specific to the 3I-genotype (C>T 7614) were strongly associated with the geographical origin of the patient. For example, the genotype C54 [SNP7614(C)/27-5(5)/12-5(4)] was associated with the Atlantic region while the genotype T54 [SNP7614(T)/27-5(4)/12-5(5) = SNP sub-type 3I] was found in Andean region. A third genotype, C64 [SNP7614(C)/27-5(6)/12-5(4) = SNP-type 1] was found mostly in between both regions (Cardona-Castro et al., 2013). Later, the same authors showed a genetic association between the strain genotype and the ancestral origin of leprosy patients from Colombia with C54 associated with African lineage, while T54 was more prevalent in patients with European ancestry (Cardona-Castro et al., 2015). WGS has not been performed .

Mycobacterium leprae genotypes also appear to have a specific distribution in the African continent. The genotype 4N followed by 4O are restricted to West Africa (Niger, Mali, Guinea-Conakry, Benin) while the genotype 4N/O was found only in Niger. Whereas the 2E, 2F and 2H strains are present in East Africa, including Ethiopia (2E, 2F and 2H) and Malawi (2E) (Benjak et al., 2018; Monot et al., 2009). Strains from SNP-types 1 and 2 also have been reported in the Democratic Republic of the Congo (Reibel et al., 2015) while the SNP-subtype 3I was reported in Morocco and Egypt as well as 3L in Egypt (Monot et al., 2009; Neukamm et al., 2020). The genotype 1D-2 was found in one sample from Niger and Congo, while the new genotype 1D-Malagasy is reported in Malawi, Madagascar and the Comoros (Avanzi et al., 2020a). Little information is available on local transmission and for samples from North, Central and South Africa, as well as on the VNTR diversity.

Limited genotyping data also is available from some other countries in South America (Singh et al., 2011), Nepal, Pakistan (Benjak et al., 2018) and French territories (Reibel et al., 2015).

However, these studies are impaired by the lack of information regarding the exact origin of the samples or the number of samples was too low to properly represent the genetic diversity in the entire country.

3.2. *Mycobacterium leprae* evolution and global distribution

3.2.1. From molecular paleoepidemiology to paleogenomics

Paleoepidemiology, the epidemiological study of disease in ancient times, can provide clues to understand the nature of infectious diseases and give a more comprehensive picture of the emergence, evolution, and spread of bacterial pathogens over history. For leprosy, this area of research is of great interest since the dynamics of *M. leprae* transmission is still not fully resolved. Besides, leprosy is one of the oldest infection scourging the world with osteological evidence pointing as early as 2000 years before the common era (B.C.E.) in India (Robbins et al., 2009) and molecular evidence of *M. leprae* infection in human remains from the 1st century common era (C.E.) has been found in Jerusalem (Witas et al., 2015). In Europe, the disease was historically believed to be introduced by the armies of Alexander the Great returning from India (~ the 4th century B.C.E.). However, the genomic characterisation has effectively contradicted this hypothesis, since the strains identified in medieval European skeletons mostly belong to the genotype 3I thereby pointing to the silk-road link between Europe and China, whereas most of the strains in India represent the SNP-type 1 which have never been identified in European skeletons.

Leprosy was highly endemic throughout Europe until the 13th century, as exemplified by the large number of leprosaria during this period (Schuenemann et al., 2013a). However, there was a sharp decline in leprosy in Europe after 14th century and reporting few cases by the 16th century, while other infectious diseases such as tuberculosis remained (Donoghue et al., 2018). The exact reasons behind this disappearance are unknown but several hypotheses have been proposed, including the loss of virulence of the bacterium following the co-evolution with humans (Heesterbeek et al., 2015; Stone et al., 2009). Molecular studies on ancient DNA are technically complex mainly because of the low amount and poor quality of the DNA. However, early in the nineties, researchers were able to identify *M. leprae* DNA on archaeological remains (Rafi et al., 1994) and a decade later for the first genotyping studies (Monot et al., 2005; Taylor et al., 2006). Since 2010, VNTR-typing was applied in two studies using ancient *M. leprae* DNA (Taylor et al., 2013; Taylor and Donoghue, 2011) while SNP-typing and SNP-

subtyping were reported in another three (Suzuki et al., 2010; Taylor and Donoghue, 2011; Van Dissel et al., 2019) and five studies (Donoghue et al., 2015; Inskip et al., 2017, 2015; Meffray et al., 2020, 2020; Taylor et al., 2013), respectively. Samples were collected mainly from human remains in Europe (Czech Republic, Denmark, Hungary, Italy, Turkey, United Kingdom) while only a few reported cases outside Europe with Japan (n=1), Suriname (n=1), and Uzbekistan (n=1).

In 2013, using an array-based enrichment method, WGS of *M. leprae* strains from medieval Northern Europe (Denmark (n=2), Sweden (n=1) and United Kingdom (n=4)) were obtained with good coverage dated from the 10th to the 14th century (Mendum et al., 2014; Schuenemann et al., 2013a). Later, six additional strains from Denmark from the same period were sequenced, one additional from UK following by three strains from Southern Europe, Italy (7th century; n=1), Hungary (7th-8th centuries; n=1), Czech Republic (9th-10th centuries; n=1) (Schuenemann et al., 2018). Recently, the oldest sequenced *M. leprae* genome was isolated from an Egyptian mummy and dates from the 2nd century B.C.E. (Neukamm et al., 2020). The comparative genomics of *M. leprae* strains from modern samples and ancient remains demonstrated that the mediaeval *M. leprae* strains do not differ significantly from the ones currently present in endemic countries (Schuenemann et al., 2013a, 2018). This indicates that it is very unlikely that the decline of leprosy in Europe during Middle Age is attributed to loss of virulence of the strains and that some other host-genetic factors or socioeconomic changes in post-plague era in Europe might have played a major role. It also provides a strong molecular evidence for the European origin of leprosy in the Americas through colonisation in the post-Columbian era (Schuenemann et al., 2013a). Thus, study of ancient medieval genomes of *M. leprae* has provided valuable insights into the spread of leprosy along with ancient human migration, the evolution of the leprosy bacillus and its origin.

3.2.2. Large scale population genomics and evolutionary model: where does *M. leprae* come from?

Analysis of informative positions deciphered from the first *M. leprae* genomes supported the hypothesis that leprosy originated in East Africa (SNP type 2) or in India (SNP type 1), from where it spread to Europe and Asia via trade routes (Monot et al., 2005). Later, using more informative sites, Monot *et al.* suggested that the *M. leprae* ancestor was probably an intermediate between the SNP type 2 and SNP type 3 (Monot et al., 2009). In 2013, comparison

of modern and ancient genomes also revealed that the 3K sub-type, composed of two modern strains from China and New Caledonia, forms a distinct branch in the *M. leprae* phylogeny, called branch 0 (Schuenemann et al., 2013b), which was estimated to have diverged from the most common ancestor around the first millennium B.C.E. (**Figure 2**). The 3K genotype has mainly been found in modern samples from China, Japan, Turkey and Iran (Monot et al., 2009; Weng et al., 2013a) as well as in ancient isolates from Europe (Donoghue et al., 2015). Very recently, the overall phylogeographic picture was refined by comparison of the genomes of 154 *M. leprae* strains derived from 25 countries and revealed nine distinct lineages or branches of *M. leprae* and subdivision of some of the 16 SNP subtypes previously described (Benjak et al., 2018). A set of 235 SNPs and 25 InDels were lineage-specific and could be used as markers for future genotyping schemes (Benjak et al., 2018). Genome sequencing of additional 3K strains from the Pacific Islands, Japan and medieval Europe identified a new distinct genotype named 3K-1 and confirmed the ancestry of the branch 0, also called the 3K-0 genotype. Bayesian inference revealed that the most recent common ancestor to all *M. leprae* strains was circulating between 2000 and 4000 years B.C.E. (Schuenemann et al., 2018). Additionally, the data suggested that the distribution of this lineage probably took place along the Silk Road. The models advocate an origin of leprosy in Western Eurasia (model 1), or an origin in East Asia and the Middle East with a previous introduction into Europe during antiquity and sooner (model 2) (Schuenemann et al., 2018). However, only few strains from Europe and East Asia have been sequenced and these hypotheses might be confirmed or challenged with the addition of genomes from Eurasia including Middle East. A phylogeographic tree based on 263 *M. leprae* isolates from 34 countries is shown in **Figure 2** and updates the data presented by Benjak *et al.* (Benjak et al., 2018).

3.3. A new leprosy-causing species

Leprosy had always been exclusively associated with *M. leprae* until 2008, when a new *Mycobacterium* species was discovered in two patients originating from Mexico (Han et al., 2008). Sequencing of more than 20 genes, including 16S rRNA, *rpoB* and *rpoT*, revealed that the overall level of nucleotide identity between the new species and *M. leprae* is 90.9%, (Han et al., 2009, 2008). In 2015, this observation was confirmed by WGS (Han et al., 2015; Singh et al., 2015). Strikingly, despite their considerable genetic divergence, clinical outcomes are similar, and it is not possible to distinguish between *M. leprae* and *M. lepromatosis* infection without molecular tests. Infections with either *M. leprae* or *M. lepromatosis* are equally treatable using standard MDT (Virk et al., 2017). In one study, genetic differences in position

54 of the *folP1* drug resistance determining region involved in resistance to the bacteriostatic drug dapsone was described when comparing *M. lepromatosis* and *M. leprae* (Kai et al., 2016). However, additional investigations are required to understand the polymorphism results in a phenotypic difference on dapsone susceptibility.

Additionally, *M. lepromatosis,* like *M. leprae,* remains uncultivated *in vitro* (Han et al., 2008) but the bacterium was recently isolated in mouse footpads, representing an invaluable resource for further screenings and investigations of this pathogen (Sharma et al., 2019). In 2019, Sharma *et al.* identified by WGS a unique repetitive region named RLPM to *M. lepromatosis*, on which basis a specific and sensitive real-time quantitative PCR assay was developed and validated (Sharma et al., 2019). Using RLPM, the authors confirmed the presence of the pathogen in 40% of leprosy cases (15/36) in Mexico and 4% in the USA (3/72) and the existence of co-infection (11%) with *M. leprae* in Mexico (Sharma et al., 2019).

From the available data, it seems that *M. lepromatosis* is distributed mainly in Central America, especially Mexico, with sporadic reports in other countries (Sharma et al., 2019). Indeed, *M. lepromatosis* also was detected in *Sciurius vulgaris* (red squirrel) in the British Isles (Scotland, Ireland and England) among animals with or without leprosy-like lesions **[section 3.4.3.1]** (Avanzi et al., 2016b; Meredith et al., 2014). Additional investigations failed to identify the pathogen in other rodent species in other countries (Schilling et al., 2019; Tió-Coma et al., 2020b) or among armadillos in the United States (Sharma et al., 2019). Similar to *M. leprae*, comparative genomics of the human-derived Mexican strain and red squirrel-derived British *M. lepromatosis* strains revealed limited genetic variations. Since no indigenous leprosy infections are reported among humans from the British Isles, the infections among red squirrels appears to present little public health risk.

3.4. Risk factors associated with leprosy development and transmission

Transmission of leprosy bacilli is thought to be mainly from person to person through respiratory routes with bacilli crossing through the nasal mucosae or damaged skin. There is a higher risk for leprosy among household contacts and people in close surroundings of active cases, especially index cases with lepromatous leprosy (Romero-Montoya et al., 2017). The existence of animal reservoirs (Oliveira et al., 2019), the presence of viable *M. leprae* in environmental samples (Chakrabarty and Dastidar, 2001) and newly detected patients with no

tangible direct source of infection (Cusini et al., 2017; Ezzedine et al., 2009; Fern et al., 2019; Musso et al., 2019; Truman and Fine, 2010) has led to the idea that additional non-human reservoirs of *M. leprae* might exist and several investigations were launched in the last decade. Molecular epidemiology is a powerful tool which should help shed new light on this important issue.

3.4.1. Inter- and intra-host variability of *Mycobacterium leprae*

3.4.1.1. Recurrence of leprosy

Recurrence of an infectious disease may occur from reinfection with a different strain of the pathogen, or regrowth of the same strain from the initial episode following effective treatment, also known as relapse. Differentiation between relapse and reinfection is of particular importance for endemic areas where convalescent cases might continue to be exposed to the pathogen (Oskam et al., 2008). Besides, both are epidemiological events with different meanings. While the relapse rate is an indicator of treatment efficiency directly linked to drug-resistance, persister infections or treatment failure; reinfection is an indicator of active transmission in the area (da Silva Rocha et al., 2011).

Leprosy recurrence is observed in many parts of the world but at varying, though low rates (Guerrero-Guerrero et al., 2012; Maghanoy et al., 2011; Shen et al., 2015; WHO, 2019). The importance of reinfection is difficult to estimate, though it is likely to play a greater role in high endemic areas than others (da Silva Rocha et al., 2011). Regardless, , any recurrent case is usually characterized by default as relapse, because conventional bacteriological methods cannot differentiate relapse from reinfection (Regional Office for South-East Asia, 2017).

There is no evidence that people gain protection against leprosy after their first episode of disease (Gelber et al., 2004). This incomplete protection might have implications in disease treatment management, the development of possible vaccines and our understanding of disease progression.

Molecular evidence for the possibility of reinfection was first shown in a patient from India (Lavania et al., 2011). Using a combination of 11 VNTR loci, Lavania and colleagues investigated the recurrence of disease among two patients who were released from therapy but presented with disease again three years later. One of them showed little differences in their

VNTR alleles suggesting the same original strain had manifest a relapse infection. While the other showed considerable difference in VNTR alleles, suggesting a different strain was manifesting the disease, and was potentially acquired by reinfection. .Da Silva Rocha and colleagues subsequently reported molecular evidence suggestive of reinfection using a combination of VNTRs and SNP-typing (da Silva Rocha et al., 2011) and identified reinfection as the cause of five out of seven relapse cases in Brazil after nine to thirteen years post primary MDT. In a more recent study on patients at a village near a former colony in the Prata in Brazil, four patients were shown to be relapses cases when their recurrent disease was assessed after four year interval using a SNP- and VNTR-typing scheme (Rosa et al., 2019).

In 2016, a study in Guinea Conakry showed that *M. leprae* strains from different patients originating from the same village or family might differ by only by as little as one uninformative SNP at genome level (Avanzi et al., 2016a). Therefore, high-resolution methods likely are needed to confirm a relapse or reinfection with a similar strain. Investigating three recurrent cases in Brazil using WGS showed that one patient presented *M. leprae* strains with different genotypes at both episodes and suggested that he was reinfected in the four year following treatment success. The distinction between relapse and reinfection with a similar strain was more challenging for the two other cases since the strains from the two episodes differed by two and zero SNPs, respectively, as well as little as 1 VNTR both cases. (Stefani et al., 2017). Combined with the clinical history of the patient, relapse was concluded in both cases. The main drawback of this study was that the genetic background of the strains circulating in the same area or household as these patients was not available for comparison with the genetic diversity observed in the strains from the two episodes. Adding complexity to the picture, the possibility of polyclonal or mixed-infection with different strains at a single time-point has been showed in *M. tuberculosis* infection and others pathogens but has so far not been observed or carefully investigated for *M. leprae* (Cohen et al., 2012).

3.4.1.2. Multi-case family and role of household contacts in disease transmission

Soon after the development of genotyping methods, molecular investigations showed that most of the patients from the same family harboured similar strain genotype (Das et al., 2020; Hall and Salipante, 2010; Matsuoka et al., 2004). However, data on strains with different genotypes were also reported suggesting that patients from the same household are exposed to different

infectious sources (Das et al., 2020; Salipante and Hall, 2011). Similar results were obtained in five family cases from Brazil (Rosa et al., 2019) and India, where one patient was infected with a strain showing different genotypes when compared to the one infecting his wife and daughter (Turankar et al., 2014). In that study, soil samples collected around the different families were of same SNP-typing than the patient strains, but the presence of viable *M. leprae* or a more detailed analysis of the genome in these samples was not evaluated.

To investigate the discriminatory power of strain typing provided by SNP and VNTR typing, WGS was applied on three strains that presented the same drug-resistant mutation in *folP1* and isolated from two siblings and one other patient living in the same region in Guinea-Conakry (Avanzi et al., 2016a). Interestingly, the three strains belong to the same clader and differed by only two uninformative SNPs. The strains from the two siblings differed by only one SNP observed in the patient who was the latest to develop symptoms. The data suggested that the siblings were infected from different sources or the sibling was already infectious before the onset of symptoms. Recently, Tió-Coma and colleagues showed that strains from the same household might also present no variation in the entire genome, being strictly genetically identical suggesting a direct transmission between the index case and the family member (Tió-Coma et al., 2020a). Therefore, if possible, when VNTR and SNP profiles are identical, comparison should be done at genome level.

The incubation period of *M. leprae* varies from 5 to 20 years and patients who developed disease at close time intervals with similar strains probably imply a common source of transmission rather than a direct transmission between the two individuals especially if they are not from the same household.

Although nasal carriage does not imply infection, the presence of *M. leprae* DNA in the nose of household contacts suggests that they are at higher risk of developing the disease. Genotyping studies also reported similar observations in multi-family cases when comparing strains from individuals with no leprosy symptoms with the strain isolated in skin lesions from patient diagnosed in the same village or the same household (Das et al., 2020; Matsuoka et al., 2004; Tió-Coma et al., 2020a). When a follow-up was performed in the household harbouring *M. leprae* DNA in nasal samples and new cases were found, molecular typing revealed that the strain from the new patient in the household was similar to the index patients is most of the cases (Romero-Montoya et al., 2017; Tió-Coma et al., 2020a). Additional follow-up studies would be required to know whether the genotype of the *M. leprae* strain identified in the lesions of newly diagnosed patient was similar to the one identified in nasal muscoa samples before

the onset of symptoms. Similarly to paucibacillary patients, individuals with no symptoms are expected to carry a low amount of *M. leprae* bacilli during the incubation period and are considered to have a low impact on disease transmission between individuals. Nevertheless, the presence of viable bacilli in the nose of uninfected individual might contribute to sustain living *M. leprae* in the environment and might, therefore, also serve as sources for transmission of bacilli (Klatser et al., 1993; Romero-Montoya et al., 2017).

3.4.1.3. Intra-patient variation and mixed infection.

Assessing the variability between strains from different patients and establish thresholds to classify mixed-infection, relapse and reinfection cases required a proper understanding of the genetic evolution dynamics of *M. leprae* bacilli inside the host. The calculated substitution rate of *M. leprae* is relatively low (7.8×10^{-9} per site per year), similar to other bacteria (Benjak et al., 2018). However, this number does not represent the mutation rate ongoing under selective pressures such as host pressure, environmental changes (pH, type of cells, nutriments access…) or exposure to antibiotics (Barrick and Lenski, 2013). As an example, strains with highly deleterious mutations in the endonuclease III gene, *nth*, encoding for the first enzyme of the base excision repair pathway, were recently isolated in leprosy patients (Benjak et al., 2018). Although such mutations would be expected not to remain fixed in the population, all strains accumulated more mutations in the same period of time compared to the strains from the same genotype (**Figure 2**). Also, they were all resistant to dapsone and some were multi-drug resistant, suggesting that the selective pressure increased mutation rate and increased the diversity of the initial population.

Longitudinal studies are usually difficult to conduct in leprosy because that requires multi-sampling of patients during the course of treatment. A sampling of SSS and NS is easier to perform than that of skin biopsies, but in general they contain a lower amount of bacterial DNA. Therefore, biopsy sampling is better suited for extensive genotyping, but it much more invasive, especially for genotyping studies as part of patient follow up but sampling other tissues might be opportune when biopsy sampling is difficult. Besides, sampling on the same site during the course of treatment is practically impossible and intra-patient variation might be anticipated since SNP and VNTR differences have been observed in strains from different samples collected from the same patient; with the nose being the most variable (Lima et al., 2016; Tió-Coma et al., 2020a; Xing et al., 2009).

Mixed infection is well described in tuberculosis as a cause of treatment failure (Cohen et al., 2012; Tarashi et al., 2017). In leprosy, the first hint suggesting that multiple strains might infect individual patients came from Young et al. (Young et al., 2008), who showed a difference in VNTR genotypes of *M. leprae* in the nerves and other body sites. However, this could also have been due to "homing" of the bacilli, allowing only a subpopulation of the same strain to invade the nervous system. In addition, only three VNTR loci were evaluated in that study, including a hypervariable AT repeat that might have resulted in overestimation of genotype variability, as suggested by Fontes *et al.* (Fontes et al. 2017). Several studies reported a difference in genotype of *M. leprae* present in the nose and the skin in a considerable number of VNTRs, highly suggestive for the presence of two different strains (Fontes et al., 2017; Lima et al., 2016). However, this not necessarily represents mixed infection because *M. leprae* in the nose could be due to passive carriage. Besides using this variability to aid in definition of stringency for cluster definition, the hypothesis was raised that this might represent the natural evolution of a single strain´s genotype migrating from what is most probably the site of entry (the nasal mucosa) to the skin, being accompanied by a high number of bacterial replications, prone to error or selection of the mutants. Recently, comparison of whole genomes of *M. leprae* present in SSS and nasal swabs collected at the same time point showed few non-informative heterologous SNP positions (Tió-Coma et al., 2020a). Interestingly, few mutations are in genes previously reported as highly mutated (e;g; *ml1512; ml1750*) in *M. leprae* strains **[section 3.4.2.3]** suggesting occurrence of both mixed-infection and in-host evolution.

3.4.2. *Mycobacterium leprae* variability and pathogenicity

Like *M. tuberculosis, M. leprae* is a clonal organism with described “negligible” genetic diversity across strains which has no known phenotypic relevance (Gagneux, 2017; Monot et al., 2005). In the tuberculosis field, this perception changed with the rise of genomics, showing that strain variation modulates the virulence, the immune phenotype and plays a key role in susceptibility to antibiotics with differential rate of emergence of drug resistance and adaptation (Gagneux, 2017). The contribution of bacterial genetic diversity is now fully considered while studying the biology and epidemiology (Gagneux, 2017) and in the intervention strategies (Drobniewski et al., 2005; L et al., 2015; Nt et al., 2003) of tuberculosis. Recent advances in molecular analysis of *M. leprae* have confirmed the existence of a similar pattern for leprosy.

Early in the seventies, Shepard and colleagues observed differences in the growth of *M. leprae* strains isolated from different patients. The so-called "slow" strains differed from the "fast" by having a longer generation time and less bacteria after harvest (Shepard and McRae, 1971). They failed to associate the strain phenotype with the geographical origin and found no correlation with disease form. Similar experiments were recently performed in nine-banded armadillo (Sharma et al., 2018) and demonstrated differential growth rate when comparing the genotype 4P (Br4923 strain from Brazil) and the zoonotic genotype 3I (NHDP-63 strain from USA). Additionally, pathological examination revealed a significant increase in bacterial dissemination through the liver and spleen of animals infected with the 4P strain compared to those with the 3I strain, suggesting pathological variations between both strains. Genetically, both genotypes differ at 121 loci, including one frameshift in *ml0825* with possible repercussions on pathogenicity (Sharma et al., 2018).

Interestingly, the SNP type 4 strains also appear at a higher frequency in relapse cases and among environmental samples around Rio de Janeiro, a region with a generally higher prevalence of SNP type 3 human infections (da Silva Rocha et al., 2011)., Leprosy prevalence also is higher in the states of the North and East part of Brazil where the genotype 4 predominates. While this phenomenon could be due to social and health care differences between the states (Nery et al., 2019) it is tempting to see a possible correlation between the genotype and the pathogenicity of the strain.

3.4.3. Animal reservoirs and environmental sources of leprosy bacilli

3.4.3.1. Animal reservoirs

Humans were thought to be the only reservoir of leprosy bacilli until the infection was discovered among nine-banded armadillos (*Dasypus novemcinctus*) in the 1970's (Job, 1981; Storrs et al., 1974; Walsh et al., 1975). Infection with *M. leprae* is well established in the wild nine-banded armadillo (*Dasypus novemcinctus*) and anecdotally in some primates from Africa (Balamayooran et al., 2015; Donham and Leininger, 1977; Gormus et al., 1988; Leininger et al., 1980). Armadillos are found only in the western hemisphere and the disease is highly prevalent among wild armadillos in the United States. Up to 20% of the animals evidence the infection in some locales. Prevalence of the infection varies markedly over the animal's geographical range (Sharma et al., 2015) with infected animals reported most commonly in low lying areas of the Southern United States. Infected armadillos also are reported from Brazil,

Mexico, Colombia and Argentina, and French Guiana (Amezcua et al., 1984; Deps et al., 2020; Oliveira et al., 2019; Schaub et al., 2020). In the United States, leprosy is considered a zoonosis and contact with armadillos is recognized as a significant risk factor for leprosy (Truman et al., 2011). The role they may play in perpetuating leprosy in other countries is under investigation.

Based on analysis of SNP- and VNTR genotyping, armadillos in the southern United States harbour only two predominate strain types denoted as 3I-2-v1 or 3I-2-v15. The v1 strain extends throughout the southern United States, while v15 is found only in the Florida peninsula (Sharma et al., 2015). In studies examining 108 unrelated patients from the region, 66% of them were found to be infected with one of the two common armadillos strains, showing that human and animal strains are being shared zoonotically. Armadillos must have acquired the disease from humans sometime following discovery and colonization of the New World. Although transmission of the infection to the animals appears to have been a rare event, it has occurred on more than one occasion and it seems likely that additional studies of armadillo populations in the United States and elsewhere will identify additional strain-types associable with zoonotic transmission. The exact mechanism of transmission of leprosy bacilli between nine-banded armadillos and humans remains and important scientific question, but close contact with contaminated flesh or blood of infected animals appears to be an important risk factor. WGS of *M. leprae* isolated from the indigenous cases in the United States (both human and armadillo) suggests a recent clonal expansion likely originating through European colonization and Africans traded as slaves (Truman et al., 2011)**(Figure 2**).

In 2016, an additional non-human host was identified when *M. leprae* was detected in the red squirrel (*Sciurus vulgaris*) population on Brownsea Island in the South of England (Avanzi et al., 2016b). The squirrel strain was a type 3I-1 and similar to one that circulated in medieval Europe (**Figure 2**). The strain is ancestral to the one found among armadillos and it is intriguing to think that squirrels may have become infected during the medieval period and harbored bacilli since that time. Additional investigation of squirrels in other parts of the British Isles and Europe failed to detect the pathogen and autochthonous human leprosy cases have not been reported from the British Isles for some time. The Brownsea's protected status may have aided survival of the infection in this particular nidus (Schilling et al., 2019; Tió-Coma et al., 2020b). Regardless, evidence is increasing that humans are not only reservoir of leprosy bacilli, and understanding the role of these others sources in the environment could have major impact on leprosy control programs.

Since both wild animal reservoirs are infected with the *M. leprae* genotype 3I, a possible correlation between the specific strain genetic background and multi-host tropism was hypothesized (Avanzi et al., 2016b). However, Honap *et al.* recently sequenced the complete genome of *M. leprae* strains from three non-human primates, including two from West Africa and one from the Philippines (Honap et al., 2018). Interestingly, the two strains from West Africa belonged to the new genotype 4N/O, previously identified in patients from Niger and Brazil (Benjak et al., 2018), while the one from the Philippines belongs to the genotype 3K-0, also isolated in human cases (Personnal communication - Dr Truman) suggesting a geographical correlation with human strains rather than a species tropism (**Figure 2**). Additionally, Sharma and colleagues showed that armadillo can experimentally be infected with other genotypes and that exclusive infection of wild armadillos with the genotype 3I in the United States is likely the result of clonal expansion rather than adaptation to a non-human host (Sharma et al., 2018).

3.4.3.2. Other potential natural reservoirs

M. leprae is an obligate intracellular parasite. Through tedious laboratory experiments using injection of material into mouse footpads, it was shown that the bacilli could survive for limited periods of time in the environment. Early studies suggested a survival of only a couple hours on a microscope slide, a few days in dessicated sputum, and up to 46 days in wet soil. (Desikan and Sreevatsa, 1995). Using molecular detection of RNA, the presumably viable *M. leprae* have been reported in soil or water around houses of leprosy patients and animal reservoirs (Adams and Lahiri, 2016; Arraes et al., 2017; Lavania et al., 2008; Matsuoka et al., 1999; Tió-Coma et al., 2019; Turankar et al., 2019). However, whether mitochondrial or ribosomal RNA should be targeted and the relative level of RNA that might be associable with actual viability of *M. leprae* in the environment has not been determined. Importantly, the capacity of leprosy bacilli to replicate into the environment remains to be elucidated. Owing to their highly degraded genomes, Truman and Fine reasoned that replication would be unlikely, given the genetic background and environmental *M. leprae* may be only transient bacilli shed by active hosts(Truman and Fine, 2010).

Another possibility is that bacteria identified in soil or water appear there in association with protozoa or other organisms which occur there naturally and are not detected when surveying

for *M. leprae* DNA or RNA (Truman and Fine, 2010). It has been shown that *M. leprae* remains viable without losing of infectivity in free-living amoebae for 35 days, but the bacilli cannot replicate in these organisms (Chavarro-Portillo et al., 2019). Additional experimental studies conducted on a different amoebae species also suggested that encysted amoebae could help sustain ingested leprosy bacilli importance of amoebae in leprosy transmission is yet unknown (Wheat et al., 2014).

The role of biting insects in leprosy transmission has never been fully discounted and a number of studies have provided anecdotal evidence that insects may help spread the disease (Fine, 1982; Kirchheimer, 1976). Neumann and colleagues experimentally demonstrated that after feeding blood meals containing *M. leprae* the feces from *Rhodnius prolixu,*(also called the kissing bugs), contained a large amount of infectious bacilli (Neumann et al., 2016). Indeed, *M. leprae* survived and remaind infectious in the digestive tract of the kissing bugs for up to 20 days after feeding on the infected blood. Interestingly, the kissing bugs transmits *Trypanosoma cruzi*, the etiological agent of Chagas Disease among humans and are also frequently found in *Dasypus novemcinctus* armadillo burrows (Neumann et al., 2016). They could be potential vehicles species-specific and inter-species transmission of leprosy among armadillo and humans. Nevertheless, such as for free-living amoebae, solid evidence through detection of *M. leprae* in environmental samples is missing.

Ticks are also considered as a potential reservoirs since the fourties, acid-fast bacilli were observed in intestinal macerates of ticks of the genus *Amblyomma* after blood-feeding on a skin lesion of a leprosy patient (Ferreira et al., 2018). Recent evidence demonstrated the presence of *M. leprae* DNA and antigens in the midgut, ovaries, eggs and larvae of *A. sculptum*, a tick species endemic in Brazil (Martins et al., 2016). The latter is capable of inoculating *M. leprae* bacilli in the skin of rabbits during blood-feeding (Ferreira et al., 2018). However, similar as for *Rhodnius prolixu,* no strong evidence for naturally *M. leprae* infected wild reservoir of ticks has been provided.

Overall, it is evident that the natural reservoir of *M. leprae* might be larger than previously anticipated and further studies might provide ground-breaking knowledge for leprosy control. Because of the difficulty in finding naturally infected potential vectors, and whenever found, to link genotypes because of scarce amount of *M. leprae* DNA, it will be hard to use molecular epidemiology studies to support the role of other potential natural reservoirs. This is not because

they are unlikely to contribute but because of the scarcity of evidence (Holanda et al., 2017; Tió-Coma et al., 2019). However, it would be an essential tool in the next decade to link human and environmental strains.

3.5. Investigation of leprosy bacilli drug susceptibility

The evolution of drug resistance is an important concern for any infectious disease. Because leprosy bacilli cannot be cultured on artificial media in the laboratory, drug susceptibility profiling of individual cases once required a year or more to complete and involved experimental inoculation of animals and monitoring growth of bacilli under various antibiotic regimen. Widespread drug resistance profiling was not possible with such techniques. With definition of the genomic sequence of *M. leprae,* molecular based assays for drug resistance became possible. Mutations in the drug resistance determining regions (DDR) of *rpoB, folp1* and *gyrA/gyrB* have been respectively associated with rifampicin, dapsone and ofloxacin resistance (Matrat et al., 2008; Williams and Gillis, 2012; Yokoyama et al., 2012). A recent global survey for antibiotic resistance among leprosy patients showed that up to 5% of relapsing cases could be associated with emerging drug resistance (Cambau et al., 2018). Resistance to the second line drugs minocycline and clarithromycin is rarely reported and, to date, the molecular mechanisms underlying resistance to those drugs have not been investigated thoroughly for *M. leprae* (Williams and Gillis, 2012). For clofazimine, the overall mechanism of action and molecular target remains to be confirmed for leprosy bacilli, but genomic polymorphisms associated with resistance to clofazimine have been identified in other mycobacterial species (Chen et al., 2018; Williams and Gillis, 2012; Yew et al., 2017) .

3.5.1. Positive selection and compensatory mutations

The selection of additional chromosomal mutations in genes encoding for drug targets is a principal example of positive selection from environmental factors. Compensatory mutations (CM) arise to compensate for fitness costs related to deleterious effects of mutations conferring drug resistance (Borrell and Gagneux, 2009). They presumably have a role in the evolution and pathogenicity of bacteria, and have potential implications with regards to transmission (Liu et al., 2018; Merker et al., 2018). In *M. tuberculosis*, CMs have been associated with rifampicin resistance and arise often in *rpoA, rpoB* and *rpoC* (Comas et al., 2012). Rifampicin resistance is rare in leprosy and this might explain why the identification of possible CM has only been

recently investigated (Cambau et al., 2018). Mutations in *rpoA, rpoB* and *rpoC* genes were identified in one, five and, two *M. leprae* rifampicin-resistant strains, respectively (Benjak et al., 2018) with only one (in *rpoA*) described to have a compensatory effect in *M. tuberculosis*.

A longitudinal study investigating the strain diversity of a relapse patient who received 48 years of irregular treatment showed the emergence of a *rpoC* mutation (L527V) in one year interval between two biopsy sampling (Avanzi et al., 2020b). The mutation has been described in *M. tuberculosis* as a CM with a low impact on fitness (Comas et al., 2012). Nevertheless, given the chronology of appearance and the association with the *rpoB* mutation the leprosy patient, it is possible that this mutation also confers a compensatory effect among *M. leprae*. These data suggests that the compensatory effect mechanism exists in *M. leprae* and might be different compared to *M. tuberculosis*. The investigations of these effects will likely reveal greater understanding of strain pathogenicity and treatment efficiency.

3.5.2. Identification of new markers of pathogenicity and resistance

Investigation of new resistance mechanisms or influence of genetic diversity on transmission and pathogenicity are usually driven by the identification of clinical differences, phenotypic characteristics and differences on an epidemiological level being either predominance of a particular strain or lineage of the causative agent or unexpected outbreaks. For example, genomic investigations of other pathogenic mycobacteria such as *M. tuberculosis* or *M. abscessus* outbreaks usually identify a handful of mutations when comparing strains circulating before and during the outbreak (Bryant et al., 2016; Folkvardsen et al., 2018). These variations are then later validated in laboratory models (Hicks et al., 2018). For *M. leprae* infection, identification of linkage between disease outcome and genotype is complicated by the wide clinical spectrum manifest by leprosy patients as well as the time scale of symptom development and the only recent availability of high resolution molecular tools for use in such investigations.

Recently, comparative genomics of these 154 *M. leprae* genomes from different geographical areas identified several hypermutated genes, presumably under positive selection, which are believed to play a role in drug resistance, pathogenesis or adaptation of the bacterium to the host (Benjak et al., 2018). Indeed, the *in vivo* drug susceptibility testing results were not available for all strains, but using the presence of mutations in the DDR of *rpo*B, *fol*P1 and

*gyr*A as a hallmark for drug resistance; the authors identified three genes (*rib*D, *fad*D9 and *nth*) highly mutated in drug vs. drug-susceptible strains, suggesting they have direct impact on drug resistance or evolution of compensatory mechanisms. Additionally, a few genes were shown to be highly mutated, independently from the drug resistance genotype, with *ml0411* being effected most frequently. ML0411 is a serine rich antigen belonging to the Pro-Pro-Glu (PPE) family with probable role as a potent B cell and T cell stimulating antigen (Macfarlane et al., 2001; Parkash, 2011). Mutations (in total seven) in this gene where also reported by Kai and colleagues with a specific pattern of mutations in strains from Japan and Korea (Kai et al., 2013). Therefore, ML0411 mutation(s) might play a role in host-pathogen interaction that requires additional investigations. Overall, the study provided valuable new insights into genes that may play a role in virulence, pathogenicity and drug-resistance for future investigations.

4. Gaps and future applications

Molecular tools and genotyping- Traditional genotyping systems are limited for a highly clonal population such as *M. leprae*. SNP-typing uses only a small portion of the *M. leprae* genome and lacks resolution to discriminate strains from members of the same family or villages. . When used alone, VNTR-typing presents too high variability for nation-wide studies and needs fine-tuning of stringency of cluster definition that might differ in scenarios such as family case transmission, relapse or reinfection with closely related strain. The combination of both methods helps to improve this resolution but is rarely used because of technical limitations and of quantity of sample required. WGS was used in several molecular studies over the last decade. It is suitable for several types of clinical samples and can be less expensive than traditional techniques. However, there are no standardized guidelines for performing WGS or interpreting the output data.

In the last decade, there has been considerable knowledge collected on molecular epidemiology studies of *M. leprae* for some countries, while others were absent or under-represented. As example, there is limited molecular information of the *M. leprae* strain circulating in Indonesia, the third country reporting the highest number of leprosy cases worldwide (WHO, 2019). Despite improvement of the basic SNP typing system in some countries and for some genotypes (Avanzi et al., 2020a; Benjak et al., 2018; Singh et al., 2014; Tió-Coma et al., 2020a; Truman et al., 2011), a redefinition of the genotyping scheme in light of latest genomic datasets would be welcome. An interesting and cost-effective approach would then be to combine WGS and

targeted sequencing approaches to decipher specific markers of the strains circulating in a given area and at the country, city, village or family levels. Later screening of isolates by PCR would decrease the cost while keeping the level of specificity required by a clonal organism such as the leprosy bacilli.

Mycobacterium lepromatosis – Currently, the global burden of *M. lepromatosis* infection seems much less than that of *M. leprae,* but this could be partly due to fewer studies on characterization of the causative agent in leprosy patients. New epidemiological surveys with the systematic characterization following by sequencing of the strains should be conducted in Mexico and the surrounding countries, especially in suspected leprosy without detection of *M. leprae.* This should be combined with studying the presence of environmental reservoirs to identify the transmission dynamics of the bacterium. Investigation of human remains could help to retrace the origin and the spread of *M. lepromatosis,* especially in Europe where the only animal reservoir has been reported (Avanzi et al., 2016b). Additional sequencing of strains from different regions might also help to build a genotyping system similar to that of *M. leprae*.

Better understanding of strain evolution and micro-epidemiology - The low substitution rate of *M. leprae* poses limitations for short-range transmission studies, including characterization of recurrent cases as relapse or reinfection. The mechanism of relapse in the absence of drug resistance is unknown in leprosy. Apart from treatment failure and non-adherence to the treatment, the existence of more virulent or persistent strains could be the basis of this phenomenon. The recent identification of hypermutated genes converged toward the existence of such strains and systematic and large-scale studies based on WGS of isolates from relapse cases will help to identify such mechanisms. This type of investigation is also particularly relevant in a frame of post-exposure prophylaxis (PEP) studies (Barth-Jaeggi et al., 2016). Indeed, the short and long-term impact of PEP on strain diversity is unknown and is a cause of concern. Therefore, SNP and VNTR-based typing methods might backup studies in countries where PEP is implemented and the level of the genome diversity before and after intervention could represent the impact of such intervention on strain selection. On the other hand, if PEP is effective, increase of strain variability as a general picture is also imaginable because less HHC would be infected so relatively longer transmission chains would occur.

Besides, there are still uncertainties about the rate of mutation of *M. leprae* strain inside the host (longitudinal studies) under antibiotic pressure and during the development of symptoms.

Strains from family members or patients living in the same area can differ by a single mutation and the genetic variation of strains from various samples collected from the same patient can present similar or even higher variability level. The latter could arise from the natural evolution of *M. leprae* inside the host or a consequence of mixed infection or reinfection and quantification of these variations are required. The combined used of WGS and targeted sequencing with specific VNTR and SNP markers could be an asset to identify more variability and quantify it in a broad number of strains.

Environmental and animal reservoirs of the leprosy bacilli – In 2011, using molecular typing, Truman and colleagues successfully linked the animal *M. leprae* isolates from wild armadillos in the US with those causing disease in humans and suggested that leprosy is a zoonosis in the southern United States (Truman et al., 2011). In parallel, a few other studies have shown the presence of the leprosy bacilli in wild armadillos in the Americas, including Brazil (da Silva Ferreira et al., 2020; da Silva et al., 2018; Deps et al., 2020; Frota et al., 2012; Ploemacher et al., 2020) but all lacked data on the parasite´s genotype. Additionally, the few studies that exist showing the presence of *M. leprae* in soil or water, when performing genotyping, present data only to the SNP-type level (Arraes et al., 2017; Turankar et al., 2014). The identification of markers and typing procedures that allow the comparison of the strains present in human host with those in environmental and animal samples would improve our understanding about the participation of the latter in human disease.

Relation between lineages/genotypes and disease characteristics - In the tuberculosis field, the different *M. tuberculosis* lineages are either generalists or specialists, the former spread on a large scale while the latter seem restricted to certain regions of the globe (Stucki et al., 2016). This also seem to occur in leprosy, where *M. leprae* strains with particular SNP types are described more locally, such as 3M, 3J, 1B, 2G, 4O, 4P and the recently described 4N/O, 1D-Malagasy and IB-Bangladesh. On the other hand, the genotypes 1D, 3I, 4N, 3K have been detected basically on a global level, including in non-human hosts. The rarity of some SNP types might be due to strain replacement, the geographic isolation of certain populations and, importantly, under-sampling from some parts of the world (Benjak et al., 2018). Nevertheless, it is tempting to speculate that *M. leprae* strains with particular genotypes are better adapted to certain human population because of host genetics, or have become more virulent or contagious.

Virulence and transmission rates are challenging to measure because of the wide range of symptoms, long incubation times and slow progress of leprosy. There are currently only a few full genomes sequenced from these rare genotypes and there is under-sampling and genome sequencing of isolates from different parts of the world, such as Indonesia, Central Asia, South Asia, and the Middle East. As such, WGS based assessment of new and rare genotypes, together with comparison of strain characteristics *in vivo* and *in vitro* carrying different genotypes could help genetic composition with differential pathogenicity, immune response and possible treatment outcome. Still, with development of powerful molecular techniques a vast amount of new knowledge about leprosy has evolved over the last decade. While tremendous challenges exist for the future, our potential to finally decipher the many mysteries of this ancient disease in the next decade have never been better.

Bibliography

Adams, L.B., Lahiri, R., 2016. Cultivation and Viability Determination of Mycobacterium leprae, in: The International Textbook of Leprosy.

Amezcua, M.E., Escobar-Gutiérrez, A., Storrs, E.E., Dhople, A.M., Burchfield, H.P., 1984. Wild Mexican armadillo with leprosy-like infection. Int. J. Lepr. Other Mycobact. Dis. 52, 254–255.

Araujo, S., Freitas, L.O., Goulart, L.R., Goulart, I.M.B., 2016. Molecular Evidence for the Aerial Route of Infection of Mycobacterium leprae and the Role of Asymptomatic Carriers in the Persistence of Leprosy. Clin. Infect. Dis. 63, 1412–1420. https://doi.org/10.1093/cid/ciw570

Arraes, M.L.B. de M., de Holanda, M.V., Lima, L.N.G.C., Sabadia, J.A.B., Duarte, C.R., Almeida, R.L.F., Kendall, C., Kerr, L.R.S., Frota, C.C., 2017. Natural environmental water sources in endemic regions of northeastern Brazil are potential reservoirs of viable Mycobacterium leprae. Mem Inst Oswaldo Cruz 112, 805–811. https://doi.org/10.1590/0074-02760170117

Avanzi, C., Busso, P., Benjak, A., Loiseau, C., Fomba, A., Doumbia, G., Camara, I., Lamou, A., Sock, G., Drame, T., Kodio, M., Sakho, F., Sow, S.O., Cole, S.T., Johnson, R.C., 2016a. Transmission of Drug-Resistant Leprosy in Guinea-Conakry Detected Using Molecular Epidemiological Approaches. Clin Infect Dis 63, 1482–1484. https://doi.org/10.1093/cid/ciw572

Avanzi, C., Del-Pozo, J., Benjak, A., Stevenson, K., Simpson, V.R., Busso, P., McLuckie, J., Loiseau, C., Lawton, C., Schoening, J., Shaw, D.J., Piton, J., Vera-Cabrera, L., Velarde-Felix, J.S., McDermott, F., Gordon, S.V., Cole, S.T., Meredith, A.L., 2016b. Red squirrels in the British Isles are infected with leprosy bacilli. Science 354, 744–747. https://doi.org/10.1126/science.aah3783

Avanzi, C., Lecorché, E., Rakotomalala, F.A., Benjak, A., Rapelanoro Rabenja, F., Ramarozatovo, L.S., Cauchoix, B., Rakoto-Andrianarivelo, M., Tió-Coma, M., Leal-Calvo, T., Busso, P., Boy-Röttger, S., Chauffour, A., Rasamoelina, T., Andrianarison, A., Sendradsoa, F.A., Spencer, J.S., Singh, P., Dashatwar, D.R., Narang, R., Berland, J.-L., Jarlier, V., Salgado, C.G., Moraes, M.O., Geluk, A., Randrianantoandro, A., Cambau, E., Cole, S.T., 2020a. Population genomics of Mycobacterium leprae reveals a new genotype in Madagascar and Comoros. Front. Microbiol. 11. https://doi.org/10.3389/fmicb.2020.00711

Avanzi, C., Maia, R.C., Benjak, A., Nery, J.A., Sales, A.M., Miranda, A., Duppre, N.C., Brum, A.F., Silva, T.P. da, Pinheiro, R.O., Neves-Manta, F., Moreira, S.J.M., Busso, P., Sarno, E.N., Suffys, P., Cole, S.T., Moraes, M.O., 2020b. Emergence of Mycobacterium leprae rifampicin resistance evaluated by whole-genome sequencing after 48 years of irregular treatment. Antimicrobial Agents and Chemotherapy. https://doi.org/10.1128/AAC.00330-20

Balamayooran, G., Pena, M., Sharma, R., Truman, R.W., 2015. The armadillo as an animal model and reservoir host for Mycobacterium leprae. Clinics in Dermatology, Leprosy: I 33, 108–115. https://doi.org/10.1016/j.clindermatol.2014.07.001

Barbieri, R.R., Manta, F.S.N., Moreira, S.J.M., Sales, A.M., Nery, J.A.C., Nascimento, L.P.R., Hacker, M.A., Pacheco, A.G., Machado, A.M., Sarno, E.M., Moraes, M.O., 2019. Quantitative polymerase chain reaction in paucibacillary leprosy diagnosis: A follow-up study. PLOS Neglected Tropical Diseases 13, e0007147. https://doi.org/10.1371/journal.pntd.0007147

Barrick, J.E., Lenski, R.E., 2013. Genome dynamics during experimental evolution. Nat Rev Genet 14, 827–839. https://doi.org/10.1038/nrg3564

Barth-Jaeggi, T., Steinmann, P., Mieras, L., van Brakel, W., Richardus, J.H., Tiwari, A., Bratschi, M., Cavaliero, A., Vander Plaetse, B., Mirza, F., Aerts, A., 2016. Leprosy Post-Exposure Prophylaxis (LPEP) programme: study protocol for evaluating the feasibility and impact on case detection rates of contact tracing and single dose rifampicin. BMJ Open 6. https://doi.org/10.1136/bmjopen-2016-013633

Benjak, A., Avanzi, C., Singh, P., Loiseau, C., Girma, S., Busso, P., Fontes, A.N.B., Miyamoto, Y., Namisato, M., Bobosha, K., Salgado, C.G., Silva, M.B., Bouth, R.C., Frade, M.A.C., Filho, F.B., Barreto, J.G., Nery, J.A.C., Bührer-Sékula, S., Lupien, A., Al-Samie, A.R., Al-Qubati, Y., Alkubati, A.S., Bretzel, G., Vera-Cabrera, L., Sakho, F., Johnson, C.R., Kodio, M., Fomba, A., Sow, S.O., Gado, M., Konaté, O., Stefani, M.M.A., Penna, G.O., Suffys, P.N., Sarno, E.N., Moraes, M.O., Rosa, P.S., Baptista, I.M.F.D., Spencer, J.S., Aseffa, A., Matsuoka, M., Kai, M., Cole, S.T., 2018. Phylogenomics and antimicrobial resistance of the leprosy bacillus Mycobacterium leprae. Nature Communications 9, 352. https://doi.org/10.1038/s41467-017-02576-z

Blok, D.J., Vlas, S.J. de, Richardus, J.H., 2016. Finding undiagnosed leprosy cases. The Lancet Infectious Diseases 16, 1113. https://doi.org/10.1016/S1473-3099(16)30370-X

Borrell, S., Gagneux, S., 2009. Infectiousness, reproductive fitness and evolution of drug-resistant Mycobacterium tuberculosis. Int. J. Tuberc. Lung Dis. 13, 1456–1466.

Bratschi, M.W., Steinmann, P., Wickenden, A., Gillis, T.P., 2015. Current knowledge on Mycobacterium leprae transmission: a systematic literature review. Lepr Rev 86, 142–155.

Britton, W.J., Lockwood, D.N., 2004. Leprosy. The Lancet 363, 1209–1219. https://doi.org/10.1016/S0140-6736(04)15952-7

Bryant, J.M., Grogono, D.M., Rodriguez-Rincon, D., Everall, I., Brown, K.P., Moreno, P., Verma, D., Hill, E., Drijkoningen, J., Gilligan, P., Esther, C.R., Noone, P.G., Giddings, O., Bell, S.C., Thomson, R., Wainwright, C.E., Coulter, C., Pandey, S., Wood, M.E., Stockwell, R.E., Ramsay, K.A., Sherrard, L.J., Kidd, T.J., Jabbour, N., Johnson, G.R., Knibbs, L.D., Morawska, L., Sly, P.D., Jones, A., Bilton, D., Laurenson, I., Ruddy, M., Bourke, S., Bowler, I.C., Chapman, S.J., Clayton, A., Cullen, M., Daniels, T., Dempsey, O., Denton, M., Desai, M., Drew, R.J., Edenborough, F., Evans, J., Folb, J., Humphrey, H., Isalska, B., Jensen-Fangel, S., Jönsson, B., Jones, A.M., Katzenstein, T.L., Lillebaek, T., MacGregor, G., Mayell, S., Millar, M., Modha, D., Nash, E.F., O'Brien, C., O'Brien, D., Ohri, C., Pao, C.S., Peckham, D., Perrin, F., Perry, A., Pressler, T., Prtak, L., Qvist, T., Robb, A., Rodgers, H., Schaffer, K., Shafi, N., van Ingen, J., Walshaw, M., Watson, D., West, N., Whitehouse, J., Haworth, C.S., Harris, S.R., Ordway, D., Parkhill, J., Floto, R.A., 2016. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. Science 354, 751–757. https://doi.org/10.1126/science.aaf8156

Cambau, E., Saunderson, P., Matsuoka, M., Cole, S.T., Kai, M., Suffys, P., Rosa, P.S., Williams, D., Gupta, U.D., Lavania, M., Cardona-Castro, N., Miyamoto, Y., Hagge, D., Srikantam, A., Hongseng, W., Indropo, A., Vissa, V., Johnson, R.C., Cauchoix, B., Pannikar, V.K., Cooreman, E. a. W.D., Pemmaraju, V.R.R., Gillini, L., WHO surveillance network of antimicrobial resistance in leprosy, 2018. Antimicrobial resistance in leprosy: results of the first prospective open survey conducted by a WHO surveillance network for the period 2009-15. Clin. Microbiol. Infect. 24, 1305–10. https://doi.org/10.1016/j.cmi.2018.02.022

Cambri, G., Mira, M.T., 2018. Genetic Susceptibility to Leprosy—From Classic Immune-Related Candidate Genes to Hypothesis-Free, Whole Genome Approaches. Front. Immunol. 9. https://doi.org/10.3389/fimmu.2018.01674
Cardona-Castro, N., 2018. Leprosy in Colombia. Curr Trop Med Rep 5, 85–90. https://doi.org/10.1007/s40475-018-0145-7
Cardona-Castro, N., Beltrán-Alzate, J.C., Romero-Montoya, I.M., Li, W., Brennan, P.J., Vissa, V., 2013. Mycobacterium leprae in Colombia described by SNP7614 in gyrA, two minisatellites and geography. Infect Genet Evol 14, 375–382. https://doi.org/10.1016/j.meegid.2012.12.015
Cardona-Castro, N., Beltrán-Alzate, J.C., Romero-Montoya, I.M., Meléndez, E., Torres, F., Sakamuri, R.M., Li, W., Vissa, V., 2009. Identification and comparison of Mycobacterium leprae genotypes in two geographical regions of Colombia. Lepr Rev 80, 316–321.
Cardona-Castro, N., Cortés, E., Beltrán, C., Romero, M., Badel-Mogollón, J.E., Bedoya, G., 2015. Human Genetic Ancestral Composition Correlates with the Origin of Mycobacterium leprae Strains in a Leprosy Endemic Population. PLOS Neglected Tropical Diseases 9, e0004045. https://doi.org/10.1371/journal.pntd.0004045
Chakrabarty, A.N., Dastidar, S.G., 2001. Is soil an alternative source of leprosy infection? Acta Leprol 12, 79–84.
Chavarro-Portillo, B., Soto, C.Y., Guerrero, M.I., 2019. Mycobacterium leprae's evolution and environmental adaptation. Acta Tropica 197, 105041. https://doi.org/10.1016/j.actatropica.2019.105041
Chen, Y., Chen, J., Zhang, S., Shi, W., Zhang, W., Zhu, M., Zhang, Y., 2018. Novel Mutations Associated with Clofazimine Resistance in Mycobacterium abscessus. Antimicrob. Agents Chemother. 62. https://doi.org/10.1128/AAC.00544-18
Cohen, T., van Helden, P.D., Wilson, D., Colijn, C., McLaughlin, M.M., Abubakar, I., Warren, R.M., 2012. Mixed-Strain Mycobacterium tuberculosis Infections and the Implications for Tuberculosis Treatment and Control. Clin Microbiol Rev 25, 708–719. https://doi.org/10.1128/CMR.00021-12
Cole, S.T., Eiglmeier, K., Parkhill, J., James, K.D., Thomson, N.R., Wheeler, P.R., Honoré, N., Garnier, T., Churcher, C., Harris, D., Mungall, K., Basham, D., Brown, D., Chillingworth, T., Connor, R., Davies, R.M., 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, M.A., Rajandream, M.A., Rutherford, K.M., Rutter, S., Seeger, K., Simon, S., Simmonds, M., Skelton, J., Squares, R., Squares, S., Stevens, K., Taylor, K., Whitehead, S., Woodward, J.R., Barrell, B.G., 2001. Massive gene decay in the leprosy bacillus. Nature 409, 1007–1011. https://doi.org/10.1038/35059006
Colman, R.E., Mace, A., Seifert, M., Hetzel, J., Mshaiel, H., Suresh, A., Lemmer, D., Engelthaler, D.M., Catanzaro, D.G., Young, A.G., Denkinger, C.M., Rodwell, T.C., 2019. Whole-genome and targeted sequencing of drug-resistant Mycobacterium tuberculosis on the iSeq100 and MiSeq: A performance, ease-of-use, and cost evaluation. PLOS Medicine 16, e1002794. https://doi.org/10.1371/journal.pmed.1002794
Comas, I., Borrell, S., Roetzer, A., Rose, G., Malla, B., Kato-Maeda, M., Galagan, J., Niemann, S., Gagneux, S., 2012. Whole-genome sequencing of rifampicin-resistant M. tuberculosis strains identifies compensatory mutations in RNA polymerase. Nat Genet 44, 106–110. https://doi.org/10.1038/ng.1038

Comas, I., Homolka, S., Niemann, S., Gagneux, S., 2009. Genotyping of Genetically Monomorphic Bacteria: DNA Sequencing in Mycobacterium tuberculosis Highlights the Limitations of Current Methodologies. PLOS ONE 4, e7815. https://doi.org/10.1371/journal.pone.0007815

Cusini, M., Marzano, A.V., Barabino, G., Benardon, S., 2017. A case of autochthonous leprosy in an elderly Italian patient leaving in Milan province with peculiar clinical presentation. Journal of the American Academy of Dermatology 76, AB3. https://doi.org/10.1016/j.jaad.2017.04.030

da Silva Ferreira, J., de Carvalho, F.M., Vidal Pessolani, M.C., de Paula Antunes, J.M.A., de Medeiros Oliveira, I.V.P., Ferreira Moura, G.H., Truman, R.W., Peña, M.T., Sharma, R., Duthie, M.S., de Paula Souza E Guimarães, R.J., Nogueira Brum Fontes, A., NoelSuffys, P., McIntosh, D., 2020. Serological and molecular detection of infection with Mycobacterium leprae in Brazilian six banded armadillos (Euphractus sexcinctus). Comp. Immunol. Microbiol. Infect. Dis. 68, 101397. https://doi.org/10.1016/j.cimid.2019.101397

da Silva, M.B., Portela, J.M., Li, W., Jackson, M., Gonzalez-Juarrero, M., Hidalgo, A.S., Belisle, J.T., Bouth, R.C., Gobbo, A.R., Barreto, J.G., Minervino, A.H.H., Cole, S.T., Avanzi, C., Busso, P., Frade, M.A.C., Geluk, A., Salgado, C.G., Spencer, J.S., 2018. Evidence of zoonotic leprosy in Pará, Brazilian Amazon, and risks associated with human contact or consumption of armadillos. PLoS Negl Trop Dis 12, e0006532. https://doi.org/10.1371/journal.pntd.0006532

da Silva Rocha, A., Cunha dos Santos, A.A., Pignataro, P., Nery, J.A., de Miranda, A.B., Soares, D.F., Brum Fontes, A.N., Miranda, A., Ferreira, H., Boéchat, N., Novisck Gallo, M.E., Sarno, E.N., De Oliveira, M.L.W., Suffys, P.N., 2011. Genotyping of Mycobacterium leprae from Brazilian leprosy patients suggests the occurrence of reinfection or of bacterial population shift during disease relapse. J Med Microbiol 60, 1441–1446. https://doi.org/10.1099/jmm.0.029389-0

Dai, C., Ansari, A., Shih, S., Marks, V., Sharma, R., Greenwald, J., 2019. Molecular epidemiology of locally acquired Hansen's disease in Central Florida. J. Am. Acad. Dermatol. 80, 1789–1791. https://doi.org/10.1016/j.jaad.2019.01.010

Das, M., Chaitanya, V.S., Kanmani, K., Rajan, L., Ebenezer, M., 2016. Genomic diversity in Mycobacterium leprae isolates from leprosy cases in South India. Infection, Genetics and Evolution 45, 285–289. https://doi.org/10.1016/j.meegid.2016.09.014

Das, M., Diana, D., Wedderburn, A., Rajan, L., Rao, S., Horo, I., Vedithi, S.C., 2020. Molecular epidemiology and transmission dynamics of leprosy among multicase families and case-contact pairs. International Journal of Infectious Diseases 96, 172–179. https://doi.org/10.1016/j.ijid.2020.04.064

de Sousa, D.B., Souza-Santos, R., Duarte da Cunha, M., Sobral, A., 2020. Hot spots of leprosy in the endemic area of São Luís, Maranhão State, Northeastern Brazil. Journal of Infection and Public Health 13, 228–234. https://doi.org/10.1016/j.jiph.2019.08.006

Deps, P.D., Antunes, J.M., Santos, A.R., Collin, S.M., 2020. Prevalence of Mycobacterium leprae in armadillos in Brazil: A systematic review and meta-analysis. PLOS Neglected Tropical Diseases 14, e0008127. https://doi.org/10.1371/journal.pntd.0008127

Desikan, K.V., Sreevatsa, null, 1995. Extended studies on the viability of Mycobacterium leprae outside the human body. Lepr Rev 66, 287–295.

Dolinger, D.L., Colman, R.E., Engelthaler, D.M., Rodwell, T.C., 2016. Next-generation sequencing-based user-friendly platforms for drug-resistant tuberculosis diagnosis: A promise for the near future. International Journal of Mycobacteriology, Supplement: The 2nd Asian-African Congress of International Journal of Mycobacteriology, Iran 5, S27–S28. https://doi.org/10.1016/j.ijmyco.2016.09.021

Donham, K.J., Leininger, J.R., 1977. Spontaneous leprosy-like disease in a chimpanzee. J. Infect. Dis. 136, 132–136.

Donoghue, H.D., Michael Taylor, G., Marcsik, A., Molnár, E., Pálfi, G., Pap, I., Teschler-Nicola, M., Pinhasi, R., Erdal, Y.S., Velemínsky, P., Likovsky, J., Belcastro, M.G., Mariotti, V., Riga, A., Rubini, M., Zaio, P., Besra, G.S., Lee, O.Y.-C., Wu, H.H.T., Minnikin, D.E., Bull, I.D., O'Grady, J., Spigelman, M., 2015. A migration-driven model for the historical spread of leprosy in medieval Eastern and Central Europe. Infect. Genet. Evol. 31, 250–256. https://doi.org/10.1016/j.meegid.2015.02.001

Donoghue, H.D., Taylor, G.M., Mendum, T.A., R.Stewart, G., Rigouts, L., Lee, O.Y.-C., Wu, H.H.T., S.Besra, G., Minnikin, D.E., 2018. The Distribution and Origins of Ancient Leprosy. Hansen's Disease - The Forgotten and Neglected Disease. https://doi.org/10.5772/intechopen.75260

Drobniewski, F., Balabanova, Y., Nikolayevsky, V., Ruddy, M., Kuznetzov, S., Zakharova, S., Melentyev, A., Fedorin, I., 2005. Drug-resistant tuberculosis, clinical virulence, and the dominance of the Beijing strain family in Russia. JAMA 293, 2726–2731. https://doi.org/10.1001/jama.293.22.2726

Duchêne, S., Holt, K.E., Weill, F.-X., Le Hello, S., Hawkey, J., Edwards, D.J., Fourment, M., Holmes, E.C., 2016. Genome-scale rates of evolutionary change in bacteria. Microb Genom 2. https://doi.org/10.1099/mgen.0.000094

Ezzedine, K., Malvy, D., Beylot, C., Longy-Boursier, M., 2009. Autochthonous leprosy in metropolitan France presenting with a diffuse infiltration of the face and febrile illness. Int. J. Dermatol. 48, 69–72. https://doi.org/10.1111/j.1365-4632.2009.03831.x

Fern, M., Esgueva, E., Fortuno, B., Torres, J.A., Cuenca, S.M., Lechuz, J.M.G., Bayon, J.V., 2019. Are there Autochthonous Cases of Leprosy in Spain? Journal of Microbial & Biochemical Technology 11, 70–72.

Ferreira, J. da S., Souza Oliveira, D.A., Santos, J.P., Ribeiro, C.C.D.U., Baêta, B.A., Teixeira, R.C., Neumann, A. da S., Rosa, P.S., Pessolani, M.C.V., Moraes, M.O., Bechara, G.H., de Oliveira, P.L., Sorgine, M.H.F., Suffys, P.N., Fontes, A.N.B., Bell-Sakyi, L., Fonseca, A.H., Lara, F.A., 2018. Ticks as potential vectors of Mycobacterium leprae: Use of tick cell lines to culture the bacilli and generate transgenic strains. PLoS Negl Trop Dis 12, e0007001. https://doi.org/10.1371/journal.pntd.0007001

Fine, P.E.M., 1982. Leprosy: the epidemiology of a slow bacterium. Epidemiol Rev 4, 161–188. https://doi.org/10.1093/oxfordjournals.epirev.a036245

Folkvardsen, D.B., Norman, A., Andersen, Å.B., Rasmussen, E.M., Lillebaek, T., Jelsbak, L., 2018. A Major Mycobacterium tuberculosis outbreak caused by one specific genotype in a low-incidence country: Exploring gene profile virulence explanations. Sci Rep 8, 1–8. https://doi.org/10.1038/s41598-018-30363-3

Fontes, A.N.B., Gomes, H.M., Araujo, M.I. de, Albuquerque, E.C.A. de, Baptista, I.M.F.D., Moura, M.M. da F., Rezende, D.S., Pessolani, M.C.V., Lara, F.A., Pontes, M.A. de A., Gonçalves, H. de S., Lucena-Silva, N., Sarno, E.N., Vissa, V.D., Brennan, P.J., Suffys, P.N., 2012. Genotyping of Mycobacterium leprae present on Ziehl-Neelsen-

stained microscopic slides and in skin biopsy samples from leprosy patients in different geographic regions of Brazil. Memórias do Instituto Oswaldo Cruz 107, 143–149. https://doi.org/10.1590/S0074-02762012000900021
Fontes, A.N.B., Lima, L.N.G.C., Mota, R.M.S., Almeida, R.L.F., Pontes, M.A., Gonçalves, H. de S., Frota, C.C., Vissa, V.D., Brennan, P.J., Guimaraes, R.J.P.S., Kendall, C., Kerr, L.R.F.S., Suffys, P.N., 2017. Genotyping of Mycobacterium leprae for better understanding of leprosy transmission in Fortaleza, Northeastern Brazil. PLOS Neglected Tropical Diseases 11, e0006117. https://doi.org/10.1371/journal.pntd.0006117
Fontes, A.N.B., Sakamuri, R.M., Baptista, I.M.F.D., Ura, S., Moraes, M.O., Martínez, A.N., Sarno, E.N., Brennan, P.J., Vissa, V.D., Suffys, P.N., 2009. Genetic diversity of mycobacterium leprae isolates from Brazilian leprosy patients. Lepr Rev 80, 302–315.
Frade, M.A.C., Foss, N.T., 2016. Editorial Commentary: Evidences of Aerial Route of Mycobacterium leprae Infection and Doubts About Transmission and Natural Protection in Leprosy. Clin Infect Dis 63, 1421–1422. https://doi.org/10.1093/cid/ciw577
Frota, C.C., Lima, L.N.C., Rocha, A. da S., Suffys, P.N., Rolim, B.N., Rodrigues, L.C., Barreto, M.L., Kendall, C., Kerr, L.R.S., 2012. Mycobacterium leprae in six-banded (Euphractus sexcinctus) and nine-banded armadillos (Dasypus novemcinctus) in Northeast Brazil. Mem. Inst. Oswaldo Cruz 107 Suppl 1, 209–213. https://doi.org/10.1590/s0074-02762012000900029
Gagneux, S. (Ed.), 2017. Strain Variation in the Mycobacterium tuberculosis Complex: Its Role in Biology, Epidemiology and Control, Advances in Experimental Medicine and Biology. Springer International Publishing.
Gelber, R.H., Balagon, V.F., Cellona, R.V., 2004. The relapse rate in MB leprosy patients treated with 2-years of WHO-MDT is not low. Int. J. Lepr. Other Mycobact. Dis. 72, 493–500. https://doi.org/10.1489/1544-581X(2004)72<493:TRRIML>2.0.CO;2
Gillis, T., Vissa, V., Matsuoka, M., Young, S., Richardus, J.H., Truman, R., Hall, B., Brennan, P., Ideal Consortium Partners, 2009. Characterisation of short tandem repeats for genotyping Mycobacterium leprae. Lepr Rev 80, 250–260.
Gormus, B.J., Wolf, R.H., Baskin, G.B., Ohkawa, S., Gerone, P.J., Walsh, G.P., Meyers, W.M., Binford, C.H., Greer, W.E., 1988. A second sooty mangabey monkey with naturally acquired leprosy: first reported possible monkey-to-monkey transmission. Int. J. Lepr. Other Mycobact. Dis. 56, 61–65.
Goulart, I.M.B., Bernardes Souza, D.O., Marques, C.R., Pimenta, V.L., Gonçalves, M.A., Goulart, L.R., 2008. Risk and Protective Factors for Leprosy Development Determined by Epidemiological Surveillance of Household Contacts. Clin Vaccine Immunol 15, 101–105. https://doi.org/10.1128/CVI.00372-07
Groathouse, N.A., Rivoire, B., Kim, H., Lee, H., Cho, S.-N., Brennan, P.J., Vissa, V.D., 2004. Multiple polymorphic loci for molecular typing of strains of Mycobacterium leprae. J. Clin. Microbiol. 42, 1666–1672.
Guan, Q., Almutairi, T.S., Alhalouli, T., Pain, A., Alasmari, F., 2020. Metagenomics of Imported Multidrug-Resistant Mycobacterium leprae, Saudi Arabia, 2017 - Volume 26, Number 3—March 2020 - Emerging Infectious Diseases journal - CDC 26, 615–617. https://doi.org/10.3201/eid2603.190661
Guerrero-Guerrero, M.I., Muvdi-Arenas, S., León-Franco, C.I., 2012. Relapses in multibacillary leprosy patients: a retrospective cohort of 11 years in Colombia. Leprosy review.

Hall, B.G., Salipante, S.J., 2010. Molecular Epidemiology of Mycobacterium leprae as Determined by Structure-Neighbor Clustering. J Clin Microbiol 48, 1997–2008. https://doi.org/10.1128/JCM.00149-10
Han, X.Y., Mistry, N.A., Thompson, E.J., Tang, H.-L., Khanna, K., Zhang, L., 2015. Draft Genome Sequence of New Leprosy Agent Mycobacterium lepromatosis. Genome Announc 3. https://doi.org/10.1128/genomeA.00513-15
Han, X.Y., Seo, Y.-H., Sizer, K.C., Schoberle, T., May, G.S., Spencer, J.S., Li, W., Nair, R.G., 2008. A new Mycobacterium species causing diffuse lepromatous leprosy. Am. J. Clin. Pathol. 130, 856–864. https://doi.org/10.1309/AJCPP72FJZZRRVMM
Han, X.Y., Sizer, K.C., Thompson, E.J., Kabanja, J., Li, J., Hu, P., Gómez-Valero, L., Silva, F.J., 2009. Comparative Sequence Analysis of Mycobacterium leprae and the New Leprosy-Causing Mycobacterium lepromatosis. J. Bacteriol. 191, 6067–6074. https://doi.org/10.1128/JB.00762-09
Heesterbeek, H., Anderson, R.M., Andreasen, V., Bansal, S., De Angelis, D., Dye, C., Eames, K.T.D., Edmunds, W.J., Frost, S.D.W., Funk, S., Hollingsworth, T.D., House, T., Isham, V., Klepac, P., Lessler, J., Lloyd-Smith, J.O., Metcalf, C.J.E., Mollison, D., Pellis, L., Pulliam, J.R.C., Roberts, M.G., Viboud, C., Isaac Newton Institute IDD Collaboration, 2015. Modeling infectious disease dynamics in the complex landscape of global health. Science 347, aaa4339. https://doi.org/10.1126/science.aaa4339
Hicks, N.D., Yang, J., Zhang, X., Zhao, B., Grad, Y.H., Liu, L., Ou, X., Chang, Z., Xia, H., Zhou, Y., Wang, S., Dong, J., Sun, L., Zhu, Y., Zhao, Y., Jin, Q., Fortune, S.M., 2018. Clinically prevalent mutations in Mycobacterium tuberculosis alter propionate metabolism and mediate multidrug tolerance. Nature Microbiology 3, 1032–1042. https://doi.org/10.1038/s41564-018-0218-3
Holanda, M.V. de, Marques, L.E.C., Macedo, M.L.B. de, Pontes, M.A. de A., Sabadia, J.A.B., Kerr, L.R.F.S., Almeida, R.L.F., Frota, C.C., Holanda, M.V. de, Marques, L.E.C., Macedo, M.L.B. de, Pontes, M.A. de A., Sabadia, J.A.B., Kerr, L.R.F.S., Almeida, R.L.F., Frota, C.C., 2017. Presence of Mycobacterium leprae genotype 4 in environmental waters in Northeast Brazil. Revista da Sociedade Brasileira de Medicina Tropical 50, 216–222. https://doi.org/10.1590/0037-8682-0424-2016
Honap, T.P., Pfister, L.-A., Housman, G., Mills, S., Tarara, R.P., Suzuki, K., Cuozzo, F.P., Sauther, M.L., Rosenberg, M.S., Stone, A.C., 2018. Mycobacterium leprae genomes from naturally infected nonhuman primates. PLOS Neglected Tropical Diseases 12, e0006190. https://doi.org/10.1371/journal.pntd.0006190
Inskip, S., Taylor, G.M., Anderson, S., Stewart, G., 2017. Leprosy in pre-Norman Suffolk, UK: biomolecular and geochemical analysis of the woman from Hoxne. Journal of Medical Microbiology. https://doi.org/10.1099/jmm.0.000606
Inskip, S.A., Taylor, G.M., Zakrzewski, S.R., Mays, S.A., Pike, A.W.G., Llewellyn, G., Williams, C.M., Lee, O.Y.-C., Wu, H.H.T., Minnikin, D.E., Besra, G.S., Stewart, G.R., 2015. Osteological, Biomolecular and Geochemical Examination of an Early Anglo-Saxon Case of Lepromatous Leprosy. PLoS ONE 10, e0124282. https://doi.org/10.1371/journal.pone.0124282
Job, C.K., 1981. Leprosy--the source of infection and its mode of transmission. Lepr Rev 52 Suppl 1, 69–76.
Kai, M., Fafutis-Morris, M., Miyamoto, Y., Mukai, T., Mayorga-Rodriguez, J., Rodriguez-Castellanos, M.A., Martínez-Guzman, M.A., Matsuoka, M., 2016. Mutations in the drug resistance-determining region of Mycobacterium lepromatosis isolated

from leprosy patients in Mexico. J. Dermatol. 43, 1345–1349. https://doi.org/10.1111/1346-8138.13483
Kai, M., Nakata, N., Matsuoka, M., Sekizuka, T., Kuroda, M., Makino, M., 2013. Characteristic mutations found in the ML0411 gene of Mycobacterium leprae isolated in Northeast Asian countries. Infection, Genetics and Evolution 19, 200–204. https://doi.org/10.1016/j.meegid.2013.07.014
Kerr-Pontes, L.R.S., Montenegro, A.C.D., Barreto, M.L., Werneck, G.L., Feldmeier, H., 2004. Inequality and leprosy in Northeast Brazil: an ecological study. Int J Epidemiol 33, 262–269. https://doi.org/10.1093/ije/dyh002
Kimura, M., Sakamuri, R.M., Groathouse, N.A., Rivoire, B.L., Gingrich, D., Krueger-Koplin, S., Cho, S.-N., Brennan, P.J., Vissa, V., 2009. Rapid Variable-Number Tandem-Repeat Genotyping for Mycobacterium leprae Clinical Specimens. Journal of Clinical Microbiology 47, 1757–1766. https://doi.org/10.1128/JCM.02019-08
Kirchheimer, W.F., 1976. The role of arthropods in the transmission of leprosy. Int. J. Lepr. Other Mycobact. Dis. 44, 104–107.
Klatser, P.R., van Beers, S., Madjid, B., Day, R., de Wit, M.Y., 1993. Detection of Mycobacterium leprae nasal carriers in populations for which leprosy is endemic. J. Clin. Microbiol. 31, 2947–2951.
Kumar, A., Girdhar, A., Chakma, J.K., Girdhar, B.K., 2013. Detection of previously undetected leprosy cases in Firozabad District (U.P.), India during 2006-2009: a short communication. Lepr Rev 84, 124–127.
Kumar, B., Uprety, S., Dogra, 2016. Clinical diagnosis of leprosy, in: The International Textbook of Leprosy.
Kuruwa, S., Vissa, V., Mistry, N., 2012. Distribution of Mycobacterium leprae Strains among Cases in a Rural and Urban Population of Maharashtra, India. J. Clin. Microbiol. 50, 1406–1411. https://doi.org/10.1128/JCM.05315-11
L, T., Dc, P., Sd, A., 2015. Moxifloxacin Prophylaxis against MDR TB, New York, New York, USA. Emerg Infect Dis 21, 500–503. https://doi.org/10.3201/eid2103.141313
Lavania, M., Jadhav, R., Turankar, R.P., Singh, I., Nigam, A., Sengupta, U., 2015. Genotyping of Mycobacterium leprae strains from a region of high endemic leprosy prevalence in India. Infection, Genetics and Evolution 36, 256–261. https://doi.org/10.1016/j.meegid.2015.10.001
Lavania, M., Jadhav, R.S., Turankar, R.P., Chaitanya, V.S., Singh, M., Sengupta, U., 2013. Single nucleotide polymorphisms typing of Mycobacterium leprae reveals focal transmission of leprosy in high endemic regions of India. Clin. Microbiol. Infect. 19, 1058–1062. https://doi.org/10.1111/1469-0691.12125
Lavania, M., Katoch, K., Katoch, V.M., Gupta, A.K., Chauhan, D.S., Sharma, R., Gandhi, R., Chauhan, V., Bansal, G., Sachan, P., Sachan, S., Yadav, V.S., Jadhav, R., 2008. Detection of viable Mycobacterium leprae in soil samples: insights into possible sources of transmission of leprosy. Infect. Genet. Evol. 8, 627–631. https://doi.org/10.1016/j.meegid.2008.05.007
Lavania, M., Katoch, K., Sharma, R., Sharma, P., Das, R., Gupta, A.K., Chauhan, D.S., Katoch, V.M., 2011. Molecular typing of Mycobacterium leprae strains from northern India using short tandem repeats. Indian J Med Res 133, 618–626.
Leininger, J.R., Donham, K.J., Meyers, W.M., 1980. Leprosy in a chimpanzee. Postmortem lesions. Int. J. Lepr. Other Mycobact. Dis. 48, 414–421.
Lima, L.N.G.C., Fontes, A.N.B., Li, W., Suffys, P.N., Vissa, V.D., Mota, R.M.S., Almeida, R.L.F., Pontes, M.A., Gonçales, H.D.S., Frota, C.C., Rodrigues, L.C., Kendall, C., Kerr, L.R.F.S., 2016. Intrapatient comparison of Mycobacterium leprae by VNTR analysis in

nasal secretions and skin biopsy in a Brazilian leprosy endemic region. Lepr Rev 87, 486–500.
Liu, Q., Zuo, T., Xu, P., Jiang, Q., Wu, J., Gan, M., Yang, C., Prakash, R., Zhu, G., Takiff, H.E., Gao, Q., 2018. Have compensatory mutations facilitated the current epidemic of multidrug-resistant tuberculosis? Emerg Microbes Infect 7. https://doi.org/10.1038/s41426-018-0101-6
Lockwood, D.N., 2004. Commentary: Leprosy and poverty. Int J Epidemiol 33, 269–270. https://doi.org/10.1093/ije/dyh115
Macfarlane, A., Mondragon-Gonzalez, R., Vega-Lopez, F., Wieles, B., Pena, J. de, Rodriguez, O., Torre, R.S. y de la, Vries, R.R.P. de, Ottenhoff, T.H.M., Dockrell, H.M., 2001. Presence of Human T-Cell Responses to the Mycobacterium leprae 45-Kilodalton Antigen Reflects Infection with or Exposure to M. leprae. Clin. Diagn. Lab. Immunol. 8, 604–611. https://doi.org/10.1128/CDLI.8.3.604-611.2001
Maghanoy, A., Mallari, I., Balagon, M., Saunderson, P., 2011. Relapse study in smear positive multibacillary (MB) leprosy after 1 year WHO-multi-drug therapy (MDT) in Cebu, Philippines. Lepr Rev 82, 65–69.
Makhado, N.A., Matabane, E., Faccin, M., Pinçon, C., Jouet, A., Boutachkourt, F., Goeminne, L., Gaudin, C., Maphalala, G., Beckert, P., Niemann, S., Delvenne, J.-C., Delmée, M., Razwiedani, L., Nchabeleng, M., Supply, P., Jong, B.C. de, André, E., 2018. Outbreak of multidrug-resistant tuberculosis in South Africa undetected by WHO-endorsed commercial tests: an observational study. The Lancet Infectious Diseases 18, 1350–1359. https://doi.org/10.1016/S1473-3099(18)30496-1
Maladan, Y., Krismawati, H., Hutapea, H.M.L., Oktavian, A., Fatimah, R., Widodo, null, 2019. A new Mycobacterium leprae dihydropteroate synthase variant (V39I) from Papua, Indonesia. Heliyon 5, e01279. https://doi.org/10.1016/j.heliyon.2019.e01279
Martins, T.F., Barbieri, A.R.M., Costa, F.B., Terassini, F.A., Camargo, L.M.A., Peterka, C.R.L., de C. Pacheco, R., Dias, R.A., Nunes, P.H., Marcili, A., Scofield, A., Campos, A.K., Horta, M.C., Guilloux, A.G.A., Benatti, H.R., Ramirez, D.G., Barros-Battesti, D.M., Labruna, M.B., 2016. Geographical distribution of Amblyomma cajennense (sensu lato) ticks (Parasitiformes: Ixodidae) in Brazil, with description of the nymph of A. cajennense (sensu stricto). Parasites & Vectors 9, 186. https://doi.org/10.1186/s13071-016-1460-2
Matrat, S., Cambau, E., Jarlier, V., Aubry, A., 2008. Are All the DNA Gyrase Mutations Found in Mycobacterium leprae Clinical Strains Involved in Resistance to Fluoroquinolones? Antimicrob Agents Chemother 52, 745–747. https://doi.org/10.1128/AAC.01095-07
Matsuoka, M., Gonzalez, A.V., Estrada, I., Carreño-Martinez, C., Fafutis-Morris, M., 2009. Various genotypes of Mycobacterium leprae from Mexico reveal distinct geographic distribution. Lepr Rev 80, 322–326.
Matsuoka, M., Izumi, S., Budiawan, T., Nakata, N., Saeki, K., 1999. Mycobacterium leprae DNA in daily using water as a possible source of leprosy infection. Indian J Lepr 71, 61–67.
Matsuoka, M., Maeda, S., Kai, M., Nakata, N., Chae, G.T., Gillis, T.P., Kobayashi, K., Izumi, S., Kashiwabara, Y., 2000. Mycobacterium leprae typing by genomic diversity and global distribution of genotypes. Int. J. Lepr. Other Mycobact. Dis. 68, 121–128.
Matsuoka, M., Zhang, L., Budiawan, T., Saeki, K., Izumi, S., 2004. Genotyping of Mycobacterium leprae on the basis of the polymorphism of TTC repeats for analysis of leprosy transmission. J. Clin. Microbiol. 42, 741–745.

Meffray, A., Houriez, E., Fossurier, C., Thuet, A., Biagini, P., Ardagna, Y., 2020. Detection of Mycobacterium leprae DNA from remains of a medieval individual, Amiens, France. Clin. Microbiol. Infect. 26, 127–129. https://doi.org/10.1016/j.cmi.2019.09.011
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., Nieselt, K., Krause, J., Stewart, G.R., 2014. Mycobacterium leprae genomes from a British medieval leprosy hospital: towards understanding an ancient epidemic. BMC Genomics 15, 270. https://doi.org/10.1186/1471-2164-15-270
Mensah-Awere, D., Bratschi, M.W., Steinmann, P., Fairley, J.K., Gillis, T.P., 2015. Symposium Report: Developing Strategies to Block the Transmission of Leprosy. Lepr Rev 86, 156–164.
Meredith, A., Pozo, J.D., Smith, S., Milne, E., Stevenson, K., McLuckie, J., 2014. Leprosy in red squirrels in Scotland. Veterinary Record 175, 285–286. https://doi.org/10.1136/vr.g5680
Merker, M., Barbier, M., Cox, H., Rasigade, J.-P., Feuerriegel, S., Kohl, T.A., Diel, R., Borrell, S., Gagneux, S., Nikolayevskyy, V., Andres, S., Nübel, U., Supply, P., Wirth, T., Niemann, S., 2018. Compensatory evolution drives multidrug-resistant tuberculosis in Central Asia. Elife 7. https://doi.org/10.7554/eLife.38200
Ministério da Saúde, 2019. Taxa de prevalência de hanseníase por 10.000 habitantes. Estados e regiões, Brasil, 1990 a 2018.
Mohanty, P.S., Bansal, A.K., Naaz, F., Arora, M., Gupta, U.D., Gupta, P., Sharma, S., Singh, H., 2019. Multiple strain infection of Mycobacterium leprae in a family having 4 patients: A study employing short tandem repeats. PLoS ONE 14, e0214051. https://doi.org/10.1371/journal.pone.0214051
Monot, M., Honoré, N., Garnier, T., Araoz, R., Coppée, J.-Y., Lacroix, C., Sow, S., Spencer, J.S., Truman, R.W., Williams, D.L., Gelber, R., Virmond, M., Flageul, B., Cho, S.-N., Ji, B., Paniz-Mondolfi, A., Convit, J., Young, S., Fine, P.E., Rasolofo, V., Brennan, P.J., Cole, S.T., 2005. On the origin of leprosy. Science 308, 1040–1042. https://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., Mays, S., Watson, C., Lockwood, D., Khamispour, A., Dowlati, Y., Jianping, S., Rea, T.H., Vera-Cabrera, L., Stefani, M.M., Banu, S., Macdonald, M., Sapkota, B.R., Spencer, J.S., Thomas, J., Harshman, K., Singh, P., Busso, P., Gattiker, A., Rougemont, J., Brennan, P.J., Cole, S.T., 2009. Comparative genomic and phylogeographic analysis of Mycobacterium leprae. Nat Genet 41, 1282–1289. https://doi.org/10.1038/ng.477
Musso, D., Rovery, C., Loukil, A., Vialette, V., Nguyen, N.L., 2019. Leprosy in French Polynesia. New Microbes New Infect 29, 100514. https://doi.org/10.1016/j.nmni.2018.10.010
Nazario, A.P., Ferreira, J., Schuler-Faccini, L., Fiegenbaum, M., Artigalás, O., Vianna, F.S.L., Nazario, A.P., Ferreira, J., Schuler-Faccini, L., Fiegenbaum, M., Artigalás, O., Vianna, F.S.L., 2017. Leprosy in Southern Brazil: a twenty-year epidemiological profile. Revista da Sociedade Brasileira de Medicina Tropical 50, 251–255. https://doi.org/10.1590/0037-8682-0229-2016
Nery, J.S., Ramond, A., Pescarini, J.M., Alves, A., Strina, A., Ichihara, M.Y., Penna, M.L.F., Smeeth, L., Rodrigues, L.C., Barreto, M.L., Brickley, E.B., Penna, G.O., 2019. Socioeconomic determinants of leprosy new case detection in the 100 Million

Brazilian Cohort: a population-based linkage study. The Lancet Global Health 7, e1226–e1236. https://doi.org/10.1016/S2214-109X(19)30260-8
Neukamm, J., Pfrengle, S., Molak, M., Seitz, A., Francken, M., Eppenberger, P., Avanzi, C., Reiter, E., Urban, C., Welte, B., Stockhammer, P.W., Teßmann, B., Herbig, A., Harvati, K., Nieselt, K., Krause, J., Schuenemann, V.J., 2020. 2000-year-old pathogen genomes reconstructed from metagenomic analysis of Egyptian mummified individuals. BMC Biol 18. https://doi.org/10.1186/s12915-020-00839-8
Neumann, A. da S., Dias, F. de A., Ferreira, J. da S., Fontes, A.N.B., Rosa, P.S., Macedo, R.E., Oliveira, J.H., Teixeira, R.L. de F., Pessolani, M.C.V., Moraes, M.O., Suffys, P.N., Oliveira, P.L., Sorgine, M.H.F., Lara, F.A., 2016. Experimental Infection of Rhodnius prolixus (Hemiptera, Triatominae) with Mycobacterium leprae Indicates Potential for Leprosy Transmission. PLoS One 11. https://doi.org/10.1371/journal.pone.0156037
Nobre, M.L., Illarramendi, X., Dupnik, K.M., Hacker, M. de A., Nery, J.A. da C., Jerônimo, S.M.B., Sarno, E.N., 2017. Multibacillary leprosy by population groups in Brazil: Lessons from an observational study. PLOS Neglected Tropical Diseases 11, e0005364. https://doi.org/10.1371/journal.pntd.0005364
Nt, L., Ht, L., B, T. le, Mw, B., K, K., D, van S., 2003. Mycobacterium tuberculosis Beijing genotype and risk for treatment failure and relapse, Vietnam. Emerg Infect Dis 9, 1633–1635. https://doi.org/10.3201/eid0912.030169
Oktaria, S., Hurif, N.S., Naim, W., Thio, H.B., Nijsten, T.E.C., Richardus, J.H., 2018. Dietary diversity and poverty as risk factors for leprosy in Indonesia: A case-control study. PLoS Negl Trop Dis 12. https://doi.org/10.1371/journal.pntd.0006317
Oliveira, I.V.P. de M., Deps, P.D., Antunes, J.M.A. de P., 2019. Armadillos and leprosy: from infection to biological model. Rev Inst Med Trop Sao Paulo 61. https://doi.org/10.1590/S1678-9946201961044
Oskam, L., Dockrell, H.M., Brennan, P.J., Gillis, T., Vissa, V., Richardus, J.H., Members of the IDEAL Consortium, 2008. Molecular methods for distinguishing between relapse and reinfection in leprosy. Tropical Medicine & International Health 13, 1325–1326. https://doi.org/10.1111/j.1365-3156.2008.02134_1.x
Parkash, O., 2011. Serological detection of leprosy employing Mycobacterium leprae derived serine-rich 45 kDa, ESAT-6, CFP-10 and PGL-I: a compilation of data from studies in Indian populations. Lepr Rev 82, 383–388.
Pattyn, S.R., 1973. The problem of cultivation of Mycobacterium leprae. Bull World Health Organ 49, 403–410.
Ploemacher, T., Faber, W.R., Menke, H., Rutten, V., Pieters, T., 2020. Reservoirs and transmission routes of leprosy; A systematic review. PLOS Neglected Tropical Diseases 14, e0008276. https://doi.org/10.1371/journal.pntd.0008276
Prakoeswa, C.R.S., Rumondor, B.B., Damayanti, L., Sasmojo, M., Adriaty, D., Alinda, M.D., Wahyuni, R., Listiawan, M.Y., Agusni, I., Izumi, S., 2019. Distribution of Mycobacterium leprae genotypes from Surabaya and Bandung Clinical Isolates by Multiple Locus Variable Number of Tandem Repeat Analysis. Dermatology Reports. https://doi.org/10.4081/dr.2019.8017
Price, V.G., 2017. Factors preventing early case detection for women affected by leprosy: a review of the literature. Glob Health Action 10, 1360550. https://doi.org/10.1080/16549716.2017.1360550

Rafi, A., Spigelman, M., Stanford, J., Lemma, E., Donoghue, H., Zias, J., 1994. DNA of Mycobacterium leprae detected by PCR in ancient bone. International Journal of Osteoarchaeology 4, 287–290. https://doi.org/10.1002/oa.1390040403
Rao, P.N., Suneetha, S., 2018. Current Situation of Leprosy in India and its Future Implications. Indian Dermatol Online J 9, 83–89. https://doi.org/10.4103/idoj.IDOJ_282_17
Regional Office for South-East Asia, W.H.O., 2017. Monitoring and Evaluation Guide: Global Leprosy Strategy 2016–2020: Accelerating towards a leprosy-free world, WHO-Global Leprosy Programme. ed.
Reibel, F., Chauffour, A., Brossier, F., Jarlier, V., Cambau, E., Aubry, A., 2015. New Insights into the Geographic Distribution of Mycobacterium leprae SNP Genotypes Determined for Isolates from Leprosy Cases Diagnosed in Metropolitan France and French Territories. PLoS Negl Trop Dis 9, e0004141. https://doi.org/10.1371/journal.pntd.0004141
Richardus, J.H., Ignotti, E., Smith, C.S., 2016. Epidemiology of Leprosy, in: International Textbook of Leprosy.
Robbins, G., Tripathy, V.M., Misra, V.N., Mohanty, R.K., Shinde, V.S., Gray, K.M., Schug, M.D., 2009. Ancient Skeletal Evidence for Leprosy in India (2000 B.C.). PLoS ONE 4, e5669. https://doi.org/10.1371/journal.pone.0005669
Romero-Montoya, M., Beltran-Alzate, J.C., Cardona-Castro, N., 2017. Evaluation and Monitoring of Mycobacterium leprae Transmission in Household Contacts of Patients with Hansen's Disease in Colombia. PLOS Neglected Tropical Diseases 11, e0005325. https://doi.org/10.1371/journal.pntd.0005325
Rosa, P.S., D'Espindula, H.R.S., Melo, A.C.L., Fontes, A.N.B., Finardi, A.J., Belone, A.F.F., Sartori, B.G.C., Pires, C.A.A., Soares, C.T., Marques, F.B., Branco, F.J.D., Baptista, I.M.F.D., Trino, L.M., Fachin, L.R.V., Xavier, M.B., Floriano, M.C., Ura, S., Diório, S.M., Delanina, W.F.B., Moraes, M.O., Virmond, M.C.L., Suffys, P.N., Mira, M.T., 2019. Emergence and transmission of drug/multidrug-resistant Mycobacterium leprae in a former leprosy colony in the Brazilian Amazon. Clin. Infect. Dis. https://doi.org/10.1093/cid/ciz570
Sakamuri, R.M., Harrison, J., Gelber, R., Saunderson, P., Brennan, P.J., Balagon, M., Vissa, V., 2009a. A continuation: study and characterisation of Mycobacterium leprae short tandem repeat genotypes and transmission of leprosy in Cebu, Philippines. Lepr Rev 80, 272–279.
Sakamuri, R.M., Kimura, M., Li, W., Kim, H.-C., Lee, H., Kiran, M.D., Black, W.C., Balagon, M., Gelber, R., Cho, S.-N., Brennan, P.J., Vissa, V., 2009b. Population-Based Molecular Epidemiology of Leprosy in Cebu, Philippines. J. Clin. Microbiol. 47, 2844–2854. https://doi.org/10.1128/JCM.02021-08
Salgado, C.G., Barreto, J.G., da Silva, M.B., Goulart, I.M.B., Barreto, J.A., de Medeiros Junior, N.F., Nery, J.A., Frade, M.A.C., Spencer, J.S., 2018. Are leprosy case numbers reliable? The Lancet Infectious Diseases 18, 135–137. https://doi.org/10.1016/S1473-3099(18)30012-4
Salgado, C.G., Barreto, J.G., Silva, M.B. da, Frade, M.A.C., Spencer, J.S., 2016. What do we actually know about leprosy worldwide? The Lancet Infectious Diseases 16, 778. https://doi.org/10.1016/S1473-3099(16)30090-1
Salipante, S.J., Hall, B.G., 2011. Towards the molecular epidemiology of Mycobacterium leprae: Strategies, successes, and shortcomings. Infection, Genetics and Evolution 11, 1505–1513. https://doi.org/10.1016/j.meegid.2011.06.003

Sarkar, R., Pradhan, S., 2016. Leprosy and women. Int J Womens Dermatol 2, 117–121. https://doi.org/10.1016/j.ijwd.2016.09.001
Schaub, R., Avanzi, C., Singh, P., Paniz-Mondolfi, A., Cardona-Castro, N., Legua, P., Crespo, L., Sewpersad, K., Dávila, J.J., Barreto, J., Dwivedi, P., Morris-Wilson, H., Larrea, M.P., Talhari, C., Lahiri, R., Truman, R.W., Gozlan, R.E., Couppié, P., de Thoisy, B., 2020. Leprosy Transmission in Amazonian Countries: Current Status and Future Trends. Curr Trop Med Rep. https://doi.org/10.1007/s40475-020-00206-1
Schilling, A.-K., Avanzi, C., Ulrich, R.G., Busso, P., Pisanu, B., Ferrari, N., Romeo, C., Mazzamuto, M.V., McLuckie, J., Shuttleworth, C.M., Del-Pozo, J., Lurz, P.W.W., Escalante-Fuentes, W.G., Ocampo-Candiani, J., Vera-Cabrera, L., Stevenson, K., Chapuis, J.-L., Meredith, A.L., Cole, S.T., 2019. British Red Squirrels Remain the Only Known Wild Rodent Host for Leprosy Bacilli. Front Vet Sci 6. https://doi.org/10.3389/fvets.2019.00008
Schuenemann, V.J., Avanzi, C., Krause-Kyora, B., Seitz, A., Herbig, A., Inskip, S., Bonazzi, M., Reiter, E., Urban, C., Pedersen, D.D., Taylor, G.M., Singh, P., Stewart, G.R., Velemínský, P., Likovsky, J., Marcsik, A., Molnár, E., Pálfi, G., Mariotti, V., Riga, A., Belcastro, M.G., Boldsen, J.L., Nebel, A., Mays, S., Donoghue, H.D., Zakrzewski, S., Benjak, A., Nieselt, K., Cole, S.T., Krause, J., 2018. Ancient genomes reveal a high diversity of Mycobacterium leprae in medieval Europe. PLOS Pathogens 14, e1006997. https://doi.org/10.1371/journal.ppat.1006997
Schuenemann, V.J., Singh, P., Mendum, T.A., Krause-Kyora, B., Jäger, G., Bos, K.I., Herbig, A., Economou, C., Benjak, A., Busso, P., Nebel, A., Boldsen, J.L., Kjellström, A., Wu, H., Stewart, G.R., Taylor, G.M., Bauer, P., Lee, O.Y.-C., Wu, H.H.T., Minnikin, D.E., Besra, G.S., Tucker, K., Roffey, S., Sow, S.O., Cole, S.T., Nieselt, K., Krause, J., 2013a. Genome-wide comparison of medieval and modern Mycobacterium leprae. Science 341, 179–183. https://doi.org/10.1126/science.1238286
Schuenemann, V.J., Singh, P., Mendum, T.A., Krause-Kyora, B., Jäger, G., Bos, K.I., Herbig, A., Economou, C., Benjak, A., Busso, P., Nebel, A., Boldsen, J.L., Kjellström, A., Wu, H., Stewart, G.R., Taylor, G.M., Bauer, P., Lee, O.Y.-C., Wu, H.H.T., Minnikin, D.E., Besra, G.S., Tucker, K., Roffey, S., Sow, S.O., Cole, S.T., Nieselt, K., Krause, J., 2013b. Genome-wide comparison of medieval and modern Mycobacterium leprae. Science 341, 179–183. https://doi.org/10.1126/science.1238286
Serrano-Coll, H., Mora, H.R., Beltrán, J.C., Duthie, M.S., Cardona-Castro, N., 2019. Social and environmental conditions related to Mycobacterium leprae infection in children and adolescents from three leprosy endemic regions of Colombia. BMC Infectious Diseases 19, 520. https://doi.org/10.1186/s12879-019-4120-2
Sharma, R., Singh, P., Loughry, W.J., Lockhart, J.M., Inman, W.B., Duthie, M.S., Pena, M.T., Marcos, L.A., Scollard, D.M., Cole, S.T., Truman, R.W., 2015. Zoonotic Leprosy in the Southeastern United States. Emerg Infect Dis 21, 2127–2134. https://doi.org/10.3201/eid2112.150501
Sharma, R., Singh, P., McCoy, R.C., Lenz, S.M., Donovan, K., Ochoa, M.T., Estrada-Garcia, I., Silva-Miranda, M., Jurado-Santa Cruz, F., Balagon, M.F., Stryjewska, B., Scollard, D.M., Pena, M.T., Lahiri, R., Williams, D.L., Truman, R.W., Adams, L.B., 2019. Isolation of Mycobacterium lepromatosis and Development of Molecular Diagnostic Assays to Distinguish M. leprae and M. lepromatosis. Clin. Infect. Dis. https://doi.org/10.1093/cid/ciz1121
Sharma, R., Singh, P., Pena, M., Subramanian, R., Chouljenko, V., Kim, J., Kim, N., Caskey, J., Baudena, M.A., Adams, L.B., Truman, R.W., 2018. Differential growth of

Mycobacterium leprae strains (SNP genotypes) in armadillos. Infection, Genetics and Evolution 62, 20–26. https://doi.org/10.1016/j.meegid.2018.04.017
Shen, J., Yan, L., Sun, P., 2015. Clinical features of relapse after multidrug therapy for leprosy in China. Lepr Rev 86, 165–169.
Shepard, C.C., McRae, D.H., 1971. Hereditary Characteristic that Varies Among Isolates of Mycobacterium leprae. Infect Immun 3, 121–126.
Shin, Y.-C., Lee, Hyejon, Lee, Hyeyoung, Walsh, G.P., Kim, J.-D., Cho, S.-N., 2000. Variable Numbers of TTC Repeats inMycobacterium leprae DNA from Leprosy Patients and Use in Strain Differentiation. J. Clin. Microbiol. 38, 4535–4538.
Shinde, V., Newton, H., Sakamuri, R.M., Reddy, V., Jain, S., Joseph, A., Gillis, T., Nath, I., Norman, G., Vissa, V., 2009. VNTR typing of Mycobacterium leprae in South Indian leprosy patients. Lepr Rev 80, 290–301.
Singh, P., Benjak, A., Carat, S., Kai, M., Busso, P., Avanzi, C., Paniz-Mondolfi, A., Peter, C., Harshman, K., Rougemont, J., Matsuoka, M., Cole, S.T., 2014. Genome-wide re-sequencing of multidrug-resistant Mycobacterium leprae Airaku-3. Clinical Microbiology and Infection 20, O619–O622. https://doi.org/10.1111/1469-0691.12609
Singh, P., Benjak, A., Schuenemann, V.J., Herbig, A., Avanzi, C., Busso, P., Nieselt, K., Krause, J., Vera-Cabrera, L., Cole, S.T., 2015. Insight into the evolution and origin of leprosy bacilli from the genome sequence of Mycobacterium lepromatosis. Proc. Natl. Acad. Sci. U.S.A. 112, 4459–4464. https://doi.org/10.1073/pnas.1421504112
Singh, P., Busso, P., Paniz-Mondolfi, A., Aranzazu, N., Monot, M., Honore, N., Belone, A. de F.F., Virmond, M., Villarreal-Olaya, M.E., Rivas, C., Cole, S.T., 2011. Molecular Drug Susceptibility Testing and Genotyping of Mycobacterium leprae Strains from South America. Antimicrob. Agents Chemother. 55, 2971–2973. https://doi.org/10.1128/AAC.00201-11
Singh, P., Cole, S.T., 2011. Mycobacterium leprae: genes, pseudogenes and genetic diversity. Future Microbiol 6, 57–71. https://doi.org/10.2217/fmb.10.153
Smith, C.S., Aerts, A., Saunderson, P., Kawuma, J., Kita, E., Virmond, M., 2017. Multidrug therapy for leprosy: a game changer on the path to elimination. Lancet Infect Dis 17, e293–e297. https://doi.org/10.1016/S1473-3099(17)30418-8
Smith, C.S., Noordeen, S.K., Richardus, J.H., Sansarricq, H., Cole, S.T., Soares, R.C., Savioli, L., Aerts, A., 2014. A strategy to halt leprosy transmission. The Lancet Infectious Diseases 14, 96–98. https://doi.org/10.1016/S1473-3099(13)70365-7
Smith, W.C., Brakel, W. van, Gillis, T., Saunderson, P., Richardus, J.H., 2015. The Missing Millions: A Threat to the Elimination of Leprosy. PLOS Neglected Tropical Diseases 9, e0003658. https://doi.org/10.1371/journal.pntd.0003658
Srisungnam, S., Rudeeaneksin, J., Lukebua, A., Wattanapokayakit, S., Pasadorn, S., Mahotarn, K., Ajincholapan, null, Sakamuri, R.M., Kimura, M., Brennan, P.J., Phetsuksiri, B., Vissa, V., 2009. Molecular epidemiology of leprosy based on VNTR typing in Thailand. Lepr Rev 80, 280–289.
Stefani, M.M.A., Avanzi, C., Bührer-Sékula, S., Benjak, A., Loiseau, C., Singh, P., Pontes, M.A.A., Gonçalves, H.S., Hungria, E.M., Busso, P., Piton, J., Silveira, M.I.S., Cruz, R., Schetinni, A., Costa, M.B., Virmond, M.C.L., Diorio, S.M., Dias-Baptista, I.M.F., Rosa, P.S., Matsuoka, M., Penna, M.L.F., Cole, S.T., Penna, G.O., 2017. Whole genome sequencing distinguishes between relapse and reinfection in recurrent leprosy cases. PLOS Neglected Tropical Diseases 11, e0005598. https://doi.org/10.1371/journal.pntd.0005598

Steinmann, P., Reed, S.G., Mirza, F., Hollingsworth, T.D., Richardus, J.H., 2017. Innovative tools and approaches to end the transmission of Mycobacterium leprae. The Lancet Infectious Diseases 17, e298–e305. https://doi.org/10.1016/S1473-3099(17)30314-6

Stone, A.C., Wilbur, A.K., Buikstra, J.E., Roberts, C.A., 2009. Tuberculosis and leprosy in perspective. American Journal of Physical Anthropology 140, 66–94. https://doi.org/10.1002/ajpa.21185

Storrs, E.E., Walsh, G.P., Burchfield, H.P., Binford, C.H., 1974. Leprosy in the Armadillo: New Model for Biomedical Research. Science 183, 851–852. https://doi.org/10.1126/science.183.4127.851

Stucki, D., Brites, D., Jeljeli, L., Coscolla, M., Liu, Q., Trauner, A., Fenner, L., Rutaihwa, L., Borrell, S., Luo, T., Gao, Q., Kato-Maeda, M., Ballif, M., Egger, M., Macedo, R., Mardassi, H., Moreno, M., Tudo Vilanova, G., Fyfe, J., Globan, M., Thomas, J., Jamieson, F., Guthrie, J.L., Asante-Poku, A., Yeboah-Manu, D., Wampande, E., Ssengooba, W., Joloba, M., Henry Boom, W., Basu, I., Bower, J., Saraiva, M., Vaconcellos, S.E.G., Suffys, P., Koch, A., Wilkinson, R., Gail-Bekker, L., Malla, B., Ley, S.D., Beck, H.-P., de Jong, B.C., Toit, K., Sanchez-Padilla, E., Bonnet, M., Gil-Brusola, A., Frank, M., Penlap Beng, V.N., Eisenach, K., Alani, I., Wangui Ndung'u, P., Revathi, G., Gehre, F., Akter, S., Ntoumi, F., Stewart-Isherwood, L., Ntinginya, N.E., Rachow, A., Hoelscher, M., Cirillo, D.M., Skenders, G., Hoffner, S., Bakonyte, D., Stakenas, P., Diel, R., Crudu, V., Moldovan, O., Al-Hajoj, S., Otero, L., Barletta, F., Jane Carter, E., Diero, L., Supply, P., Comas, I., Niemann, S., Gagneux, S., 2016. Mycobacterium tuberculosis lineage 4 comprises globally distributed and geographically restricted sublineages. Nat. Genet. 48, 1535–1543. https://doi.org/10.1038/ng.3704

Sun, P.-W., Wang, L., Yu, M.-W., Gu, H., Shen, J.-P., Yan, L.-B., Zhang, G.-C., 2019. Leprosy Statistics in China, 2017. International Journal of Dermatology and Venereology 2, 1–5. https://doi.org/10.3760/cma.j.issn.2096-5540.2019.01.001

Suzuki, K., Takigawa, W., Tanigawa, K., Nakamura, K., Ishido, Y., Kawashima, A., Wu, H., Akama, T., Sue, M., Yoshihara, A., Mori, S., Ishii, N., 2010. Detection of Mycobacterium leprae DNA from Archaeological Skeletal Remains in Japan Using Whole Genome Amplification and Polymerase Chain Reaction. PLoS ONE 5, e12422. https://doi.org/10.1371/journal.pone.0012422

Tarashi, S., Fateh, A., Mirsaeidi, M., Siadat, S.D., Vaziri, F., 2017. Mixed infections in tuberculosis: The missing part in a puzzle. Tuberculosis 107, 168–174. https://doi.org/10.1016/j.tube.2017.09.004

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. Microbes Infect. 13, 923–929. https://doi.org/10.1016/j.micinf.2011.05.003

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

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. Journal of Archaeological Science 33, 1569–1579. https://doi.org/10.1016/j.jas.2006.02.008

Tió-Coma, M., Avanzi, C., Verhard, E.M., Pierneef, L., Van Hooij, A., Benjak, A., Roy, J.C., Khatun, M., Alam, K., Corstjens, P., Cole, S.T., Richardus, J.H., Geluk, A., 2020a. Genomic characterization of Mycobacterium leprae to explore transmission patterns identifies new subtype in Bangladesh. Front. Microbiol. 11. https://doi.org/10.3389/fmicb.2020.01220

Tió-Coma, M., Sprong, H., Kik, M., Dissel, J.T. van, Han, X.-Y., Pieters, T., Geluk, A., 2020b. Lack of evidence for the presence of leprosy bacilli in red squirrels from North-West Europe. Transboundary and Emerging Diseases 67, 1032–1034. https://doi.org/10.1111/tbed.13423

Tió-Coma, M., Wijnands, T., Pierneef, L., Schilling, A.K., Alam, K., Roy, J.C., Faber, W.R., Menke, H., Pieters, T., Stevenson, K., Richardus, J.H., Geluk, A., 2019. Detection of Mycobacterium leprae DNA in soil: multiple needles in the haystack. Sci Rep 9, 3165. https://doi.org/10.1038/s41598-019-39746-6

Truman, R., Fine, P.E.M., 2010. "Environmental" sources of Mycobacterium leprae: issues and evidence. Lepr Rev 81, 89–95.

Truman, R., 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. New England Journal of Medicine 364, 1626–1633. https://doi.org/10.1056/NEJMoa1010536

Truman, R.W., Krahenbuhl, J.L., 2001. Viable M. leprae as a research reagent. Int. J. Lepr. Other Mycobact. Dis. 69, 1–12.

Turankar, R.P., Lavania, M., Chaitanya, V.S., Sengupta, U., Darlong, J., Darlong, F., Siva Sai, K.S.R., Jadhav, R.S., 2014. Single nucleotide polymorphism-based molecular typing of M. leprae from multicase families of leprosy patients and their surroundings to understand the transmission of leprosy. Clin Microbiol Infect 20, O142–O149. https://doi.org/10.1111/1469-0691.12365

Turankar, R.P., Singh, V., Gupta, H., Pathak, V.K., Ahuja, M., Singh, I., Lavania, M., Dinda, A.K., Sengupta, U., 2019. Association of non-tuberculous mycobacteria with Mycobacterium leprae in environment of leprosy endemic regions in India. Infect. Genet. Evol. 72, 191–198. https://doi.org/10.1016/j.meegid.2018.11.010

Van Dissel, J.T., Pieters, T., Geluk, A., Maat, G., Menke, H.E., Tió-Coma, M., Altena, E., Laros, J.F.J., Adhin, M.R., 2019. Archival, paleopathological and aDNA-based techniques in leprosy research and the case of Father Petrus Donders at the Leprosarium 'Batavia', Suriname. International Journal of Paleopathology 27, 1–8. https://doi.org/10.1016/j.ijpp.2019.08.001

Vieira, M.C.A., Nery, J.S., Paixão, E.S., Andrade, K.V.F. de, Penna, G.O., Teixeira, M.G., 2018. Leprosy in children under 15 years of age in Brazil: A systematic review of the literature. PLOS Neglected Tropical Diseases 12, e0006788. https://doi.org/10.1371/journal.pntd.0006788

Virk, A., Pritt, B., Patel, R., Uhl, J.R., Bezalel, S.A., Gibson, L.E., Stryjewska, B.M., Peters, M.S., 2017. Mycobacterium lepromatosis Lepromatous Leprosy in US Citizen Who Traveled to Disease-Endemic Areas. Emerg Infect Dis 23, 1864–1866. https://doi.org/10.3201/eid2311.171104

Walsh, G.P., Storrs, E.E., Burchfield, H.P., Cotrell, E.H., Vidrine, M.F., Binford, C.H., 1975. Leprosy-like disease occurring naturally in armadillos. J Reticuloendothel Soc 18, 347–351.

Wang, X., Jordan, I.K., Mayer, L.W., 2015. Chapter 29 - A Phylogenetic Perspective on Molecular Epidemiology, in: Tang, Y.-W., Sussman, M., Liu, D., Poxton, I., Schwartzman, J. (Eds.), Molecular Medical Microbiology (Second Edition).

Academic Press, Boston, pp. 517–536. https://doi.org/10.1016/B978-0-12-397169-2.00029-9
Wangara, F., Kipruto, H., Ngesa, O., Kayima, J., Masini, E., Sitienei, J., Ngari, F., 2019. The spatial epidemiology of leprosy in Kenya: A retrospective study. PLOS Neglected Tropical Diseases 13, e0007329. https://doi.org/10.1371/journal.pntd.0007329
Weng, X., Vander Heiden, J., Xing, Y., Liu, J., Vissa, V., 2011. Transmission of leprosy in Qiubei County, Yunnan, China: insights from an 8-year molecular epidemiology investigation. Infect. Genet. Evol. 11, 363–374. https://doi.org/10.1016/j.meegid.2010.11.014
Weng, X., Xing, Y., Liu, J., Wang, Y., Ning, Y., Li, M., Wu, W., Zhang, L., Li, W., Heiden, J.V., Vissa, V., 2013a. 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. https://doi.org/10.1016/j.meegid.2012.12.009
Weng, X., Xing, Y., Liu, J., Wang, Y., Ning, Y., Li, M., Wu, W., Zhang, L., Li, W., Vander Heiden, J., Vissa, V., 2013b. 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. https://doi.org/10.1016/j.meegid.2012.12.009
Wheat, W.H., Casali, A.L., Thomas, V., Spencer, J.S., Lahiri, R., Williams, D.L., McDonnell, G.E., Gonzalez-Juarrero, M., Brennan, P.J., Jackson, M., 2014. Long-term Survival and Virulence of Mycobacterium leprae in Amoebal Cysts. PLoS Negl Trop Dis 8, e3405. https://doi.org/10.1371/journal.pntd.0003405
WHO, 2019. Global leprosy update, 2018: moving towards a leprosy- free world (Weekly epidemiological record No. 94 [35/36]).
WHO, W., 2015. Global leprosy update, 2014: need for early case detection (Weekly epidemiological record: No. 36).
Williams, D.L., Gillis, T.P., 2012. Drug-resistant leprosy: monitoring and current status. Lepr Rev 83, 269–281.
Witas, H.W., Donoghue, H.D., Kubiak, D., Lewandowska, M., Gładykowska-Rzeczycka, J.J., 2015. Molecular studies on ancient M. tuberculosis and M. leprae: methods of pathogen and host DNA analysis. Eur J Clin Microbiol Infect Dis 34, 1733–1749. https://doi.org/10.1007/s10096-015-2427-5
World Health Organization, 2008. Trends in the epidemiology of leprosy — Viet Nam, 1983-2006 = Tendances concernant répidémiologie de la lèpre. Weekly Epidemiological Record 83, 217–224.
Xing, Y., Liu, J., Sakamuri, R.M., Wang, Z., Wen, Y., Vissa, V., Weng, X., 2009. VNTR typing studies of Mycobacterium leprae in China: assessment of methods and stability of markers during treatment. Lepr Rev 80, 261–271.
Yew, W.W., Liang, D., Chan, D.P., Shi, W., Zhang, Y., 2017. Molecular mechanisms of clofazimine resistance in Mycobacterium tuberculosis. J Antimicrob Chemother 72, 2943–2944. https://doi.org/10.1093/jac/dkx227
Yokoyama, K., Kim, H., Mukai, T., Matsuoka, M., Nakajima, C., Suzuki, Y., 2012. Impact of amino acid substitutions in B subunit of DNA gyrase in Mycobacterium leprae on fluoroquinolone resistance. PLoS Negl Trop Dis 6, e1838. https://doi.org/10.1371/journal.pntd.0001838
Young, S.K., Ponnighaus, J.M., Jain, S., Lucas, S., Suneetha, S., Lockwood, D.N.J., Young, D.B., Fine, P.E.M., 2008. Use of Short Tandem Repeat Sequences to Study Mycobacterium leprae in Leprosy Patients in Malawi and India. PLOS Neglected Tropical Diseases 2, e214. https://doi.org/10.1371/journal.pntd.0000214

Yuan, Y., Wen, Y., You, Y., Xing, Y., Li, H., Weng, X., Wu, N., Liu, S., Zhang, S., Zhang, W., Zhang, Y., 2015. Characterization of Mycobacterium leprae Genotypes in China—Identification of a New Polymorphism C251T in the 16S rRNA Gene. PLOS ONE 10, e0133268. https://doi.org/10.1371/journal.pone.0133268

Table: Improvements in *Mycobacterium leprae* genotyping systems by comparative genomic analysis of *M. leprae* genomes from different parts of the world.

Actions	Genotype's name	Observations	References
New genotype	4N/O	Observed in clinical isolates from Brazil, Niger and in non-human primate from West Africa	(Benjak et al., 2018; Honap et al., 2018; Stefani et al., 2017)
New genotype	1D-Malagasy	Observed in clinical isolates from Madagascar, Comoros and Malawi	(Avanzi et al., 2020a)
New genotype	1B-Bangladesh	Observed in clinical isolates from Bangladesh	(Tió-Coma et al., 2020a)
New genotype	3K-1	Observed in clinical isolates from the Pacific Island such as Japan or US- islands	(Benjak et al., 2018)
Misclassification	1C	Samples with the informative 1C SNPs clustered inside the 1D or 3I genotypes	(Tió-Coma et al., 2020a)
Deeper resolution	1D-1 *vs.* 1D-2 *vs.* 1D-Malagasy	1D-1: South America and West Africa 1D-2: South and South-East Asia 1D-Malagasy:	(Avanzi et al., 2020a; Singh et al., 2014)
Deeper resolution	3I-1 *vs.* 3I-2	3I-1: medieval strains from Europe and few from Brazil 3I-2: strains from South America and the United States	(Benjak et al., 2018; Truman et al., 2011)
Deeper resolution	3I-2-v1 *vs.* 3I-2-v15	Observed in isolates from patients and armadillos in the United States	(Sharma et al., 2015; Truman et al., 2011)
Deeper resolution	*rpoT*-3/4 copies	4 copies of hexamer repeat in *rpoT* gene are found only in 3K-0 strains of *M. leprae* from	(Benjak et al., 2018)

		Japan and Korea as well as in *M. lepromatosis.*	

Legend to Figures

Figure 1: Distribution of *Mycobacterium leprae* SNP-subtypes in Brazil, India and China – the maps were drawn using the genotype information of the 1059 *M. leprae* strains published in the past decade from India (n=538) (Das et al., 2016; Kuruwa et al., 2012; Lavania et al., 2015, 2013; Mohanty et al., 2019; Turankar et al., 2014), Brazil (n=348) (Avanzi et al., 2020a; Benjak et al., 2018; Fontes et al., 2017, 2012, 2009; Holanda et al., 2017; Lima et al., 2016; Stefani et al., 2017) and China (n=173) (Weng et al., 2013b; Yuan et al., 2015) – the * represents the provinces in China where four copies of the hexamer repeat in the *rpoT* were found and is highlight in red when all 3K strains from the provinces have the 4 copies.

Figure 2. Phylogenetic tree based on genomes of 263 isolates of *Mycobacterium leprae* - The tree was build using Maximum Spanning of 263 isolates derived from 34 countries with *M. lepromatosis* as an outgroup. Animal isolates included are shown with the corresponding black image: nine-banded armadillo, non-human primates and red squirrels. The outer circle represents the different SNP-based genotypes while the inner circle represents the five main branches. The colors represent the different continents and sub-continents with the latter indicated at the end of each line. *: strains with the hypermutator genotype.

Figure 1.

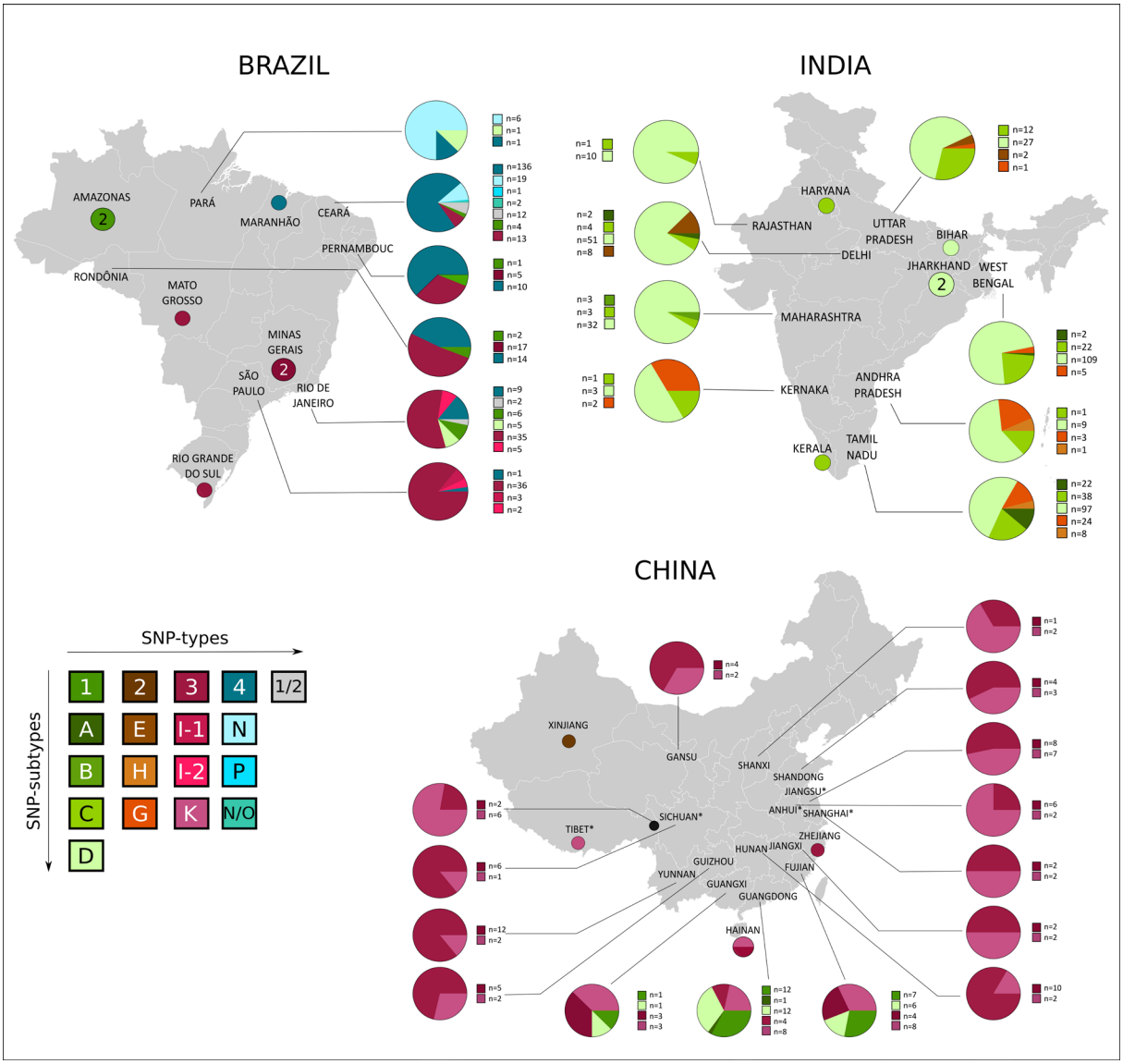
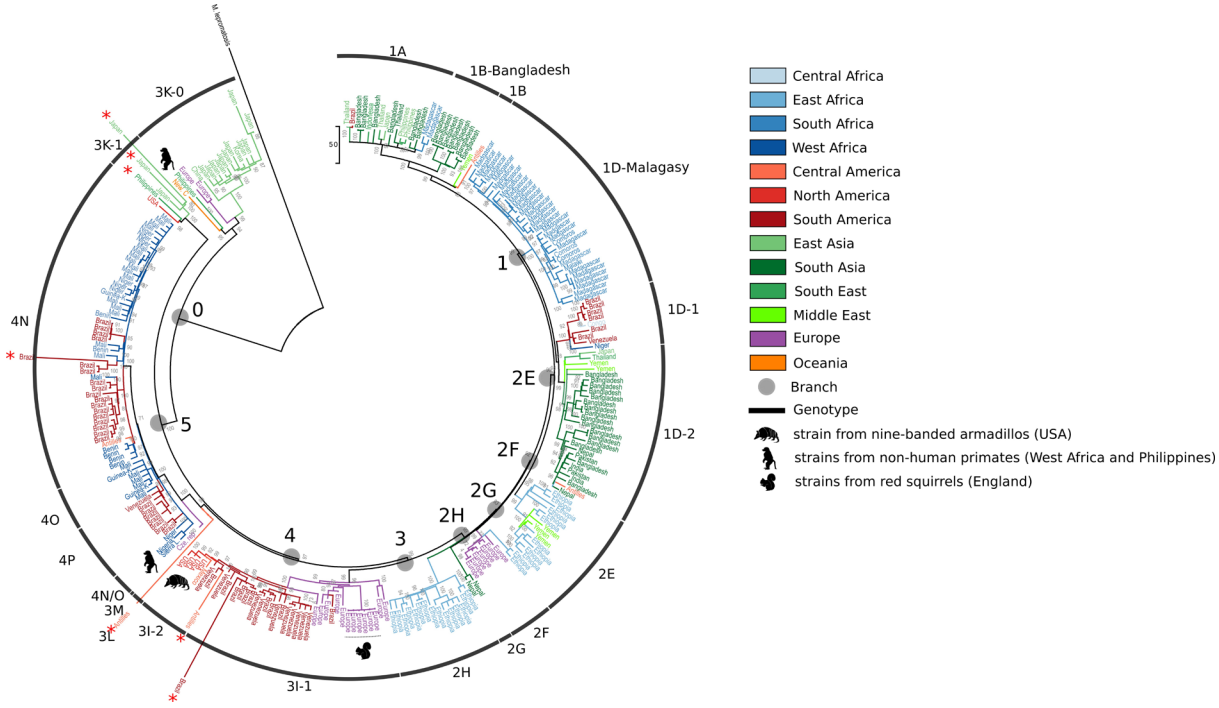


Figure 2.



Supplementary Table: List of publications related to molecular epidemiology of leprosy since 2011