

# Analysis of a nonhuman primate *Mycobacterium leprae* strain: implications for zoonotic transmission of mycobacterial pathogens

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## Background

The genus *Mycobacterium* comprises many important human pathogens such as the *Mycobacterium tuberculosis* complex (MTBC), which causes tuberculosis, and *M. leprae*, which causes leprosy. Members of the MTBC can be transmitted bidirectionally among humans and several other mammalian species including nonhuman primates. MTBC infection in nonhuman primates shows a range of manifestations- from asymptomatic infection to active tuberculosis disease. In contrast, *M. leprae* primarily infects humans and nine-banded armadillos; however, isolated cases of *M. leprae* infection have been observed in nonhuman primates such as cynomolgus macaques<sup>1</sup>, chimpanzees<sup>2-4</sup>, and sooty mangabeys<sup>5,6</sup>.

Interactions of humans with nonhuman primates, such as through zoos, sanctuaries, or due to their use for sport, entertainment, and as family pets, facilitates pathogen transmission. To assess the risk of anthroponotic as well as zoonotic transmission of mycobacterial pathogens, a broad screening of nonhuman primates living in close proximity to human populations is necessary. In tuberculosis-endemic countries such as Peru and Brazil, presence of MTBC infection as well as tuberculosis disease has been reported in captive New World primates<sup>7,8</sup>. Previous research from the Stone Laboratory has demonstrated the presence of mycobacterial infection in wild marmosets from Brazil<sup>9</sup>. However, to the best of our knowledge, no study has reported the presence of MTBC or *M. leprae* infection in wild New World primates.



Fig. 1: Sooty mangabey with leprosy<sup>6</sup>.

## Genomic analysis of the mangabey *M. leprae* strain

### Objective

To analyze the genome data for an *M. leprae* strain isolated from a naturally infected sooty mangabey.

### Methods

- Sequencing of the mangabey *M. leprae* strain using Roche 454 technology.
- Mapping of sequence reads to the *M. leprae* TN reference genome using Bowtie<sup>10</sup>.
- Quality-filtering of mapped reads and removal of PCR duplicates using SAMTools<sup>11</sup>.
- Visualization of alignments using Geneious R6<sup>12</sup>.
- Phylogenetic analyses using MEGA6<sup>13</sup>.

### Results

- The mangabey *M. leprae* strain was sequenced with 42.6-fold mean coverage and 98.8% of the genome was covered at least one-fold.
- The strain has 22 unique single nucleotide polymorphisms (SNPs). It can be classified as *M. leprae* subtype 4N based upon the presence of characteristic SNPs<sup>14</sup>.

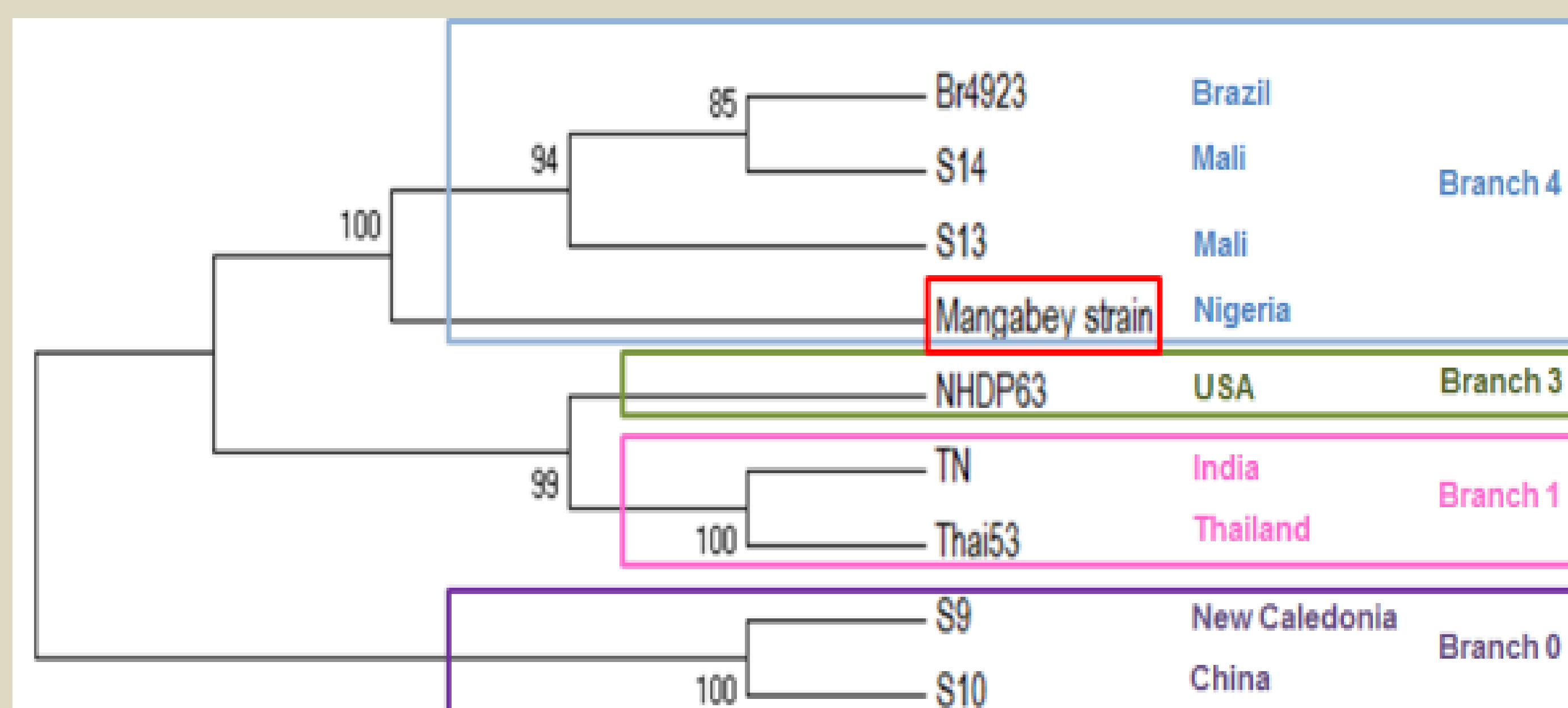


Fig. 2: Maximum Likelihood tree based on 497 variable nucleotide positions across the *M. leprae* genome. The tree was generated using the HKY model determined to be the best model of evolution based on MEGA's model test. Bootstrap support was generated using 1000 replicates. Geographic origin of each strain is given adjacent to the name of the strain. The branches are represented by different colors. Genomic data for human *M. leprae* strains used in this phylogeny were obtained from GenBank and SRA.

## Discussion

- The mangabey *M. leprae* strain is closely related to human *M. leprae* Branch 4 strains, which are primarily found in West Africa<sup>14</sup>.
- The mangabey was imported from Nigeria to the USA for research purposes in the 1970s and never came into contact with a known source of leprosy<sup>5</sup>.
- Therefore, the mangabey probably acquired leprosy from a human in Nigeria prior to being shipped to the USA.
- This suggests that *M. leprae* might be transmitted from humans to nonhuman primates in close contact especially in leprosy-endemic countries.

## Future Work

- Sequencing the genomes of *M. leprae* strains from a naturally infected chimpanzee and cynomolgus macaque.

## Screening of New World primates for mycobacteria

### Objective

To screen wild *Saguinus imperator* and *S. fuscicollis* individuals (tamarins) from Peru for the presence of mycobacterial infection.

### Methods

- Cheek swab collection from approximately 100 *Saguinus* individuals from the Los Amigos biological field station in southeastern Peru.
- DNA extraction of 50 samples using a standard phenol-chloroform extraction protocol<sup>15</sup>.
- Testing for mycobacterial genes *rpoB* (common to all mycobacteria), IS6110 (MTBC-specific), and RLEP and 85B (*M. leprae*-specific) using quantitative PCR assays (qPCR)<sup>16-18</sup>.
- Library preparation of qPCR-positive samples<sup>19</sup>.
- Targeted enrichment for mycobacterial genes *rpoB*, *gyrA*, and *gyrB* and sequencing using an Illumina MiSeq<sup>20</sup>.
- Mapping of quality-filtered sequence reads to the *M. tuberculosis* H37Rv reference genome using BWA<sup>21</sup>.

### Results

- Eight out of 50 tamarin DNA extracts (16%) tested positive for the *rpoB1* qPCR assay.
- None of the samples tested positive for the MTBC-specific qPCR assays (*rpoB2* and IS6110) or the *M. leprae*-specific qPCR assays (85B and *rlep*).
- Across all eight samples, very few reads (ranging from zero to 31) mapped to the *M. tuberculosis* H37Rv reference genome.

Samples tested	No. of samples positive for qPCRs targeting mycobacterial genes				
	<i>rpoB1</i>	<i>rpoB2</i>	IS6110	85B	<i>rlep</i>
50	8	0	0	0	0

## Discussion

- Eight tamarin DNA extracts tested positive for the *rpoB1* qPCR assay, which targets the mycobacterial *rpoB* gene. None of the samples tested positive for the *rpoB2* qPCR assay, which targets a region of the *rpoB* gene specific to the MTBC.
- Sequence data for three common mycobacterial genes could not be used to identify the species due to insufficient coverage of the targeted regions.
- It is likely that the mycobacterial species present in these samples is a member of the genus *Mycobacterium* that is related to the MTBC, but is not a member of the complex.

## Future Work

- Testing the remaining 50 tamarin samples for the presence of mycobacterial pathogens.
- Screening New World primate samples from Costa Rica.