Supplementary Text S1 - Detailed methods

Consent/ethics

The study (access to patients' clinical data) was approved by the NHS Health Research Authority (HRA) and Health and Care Research Wales (HCRW) (REC reference 21/HRA/2554).

Culturing, DNA extraction and sequencing

Sputum samples were grown in BBL MGIT media (Becton Dickinson; BD) in a BACTEC MGIT 960 (BD) culture system until the system indicated likely Mycobacterial growth. NTM species confirmation was performed using the GenoTypeMycobacterium CM VER 2.0 (Hain Lifescience) System. Confirmed MAC cultures were regrown from bead stock cultures in BBL MGIT media (BD) in a BACTEC MGIT 960 (BD) culture system until the system indicated growth. In the absence of growth, DNA was extracted from the bead stock. DNA extractions were performed as previously described (https://dx.doi.org/10.17504/protocols.io.bf28jqhw). A total of 1189 DNA extracts were sequenced by the core pipeline teams at the Wellcome Sanger Institute.

Clinical data pertaining to patients from whom NTM cultures were isolated were collected from electronic health records at the RBH. Data included patients' sex, age at the time of first positive NTM culture, height, weight, lung function test results, comorbidities, medication history and date of death (where applicable). Anonymization was undertaken by removing personal data, including patients' hospital numbers, prior to analysis.

Sequence QC, mapping and phylogenetics

Basic quality control metrics for the raw sequence data were generated using FastQC v0.11.9 [1]. Sequence reads with similarity to *Mycobacterium* species were identified using Kraken v0.10.6 [2] and Bracken v1.0 [3]. Samples with < 70% reads mapping to a *Mycobacterium* species were excluded from further analyses (n = 116). Seven isolates not belonging to the MAC (*M. abscessus, M. chelonae, M. simiae)* were removed from the dataset. Sequence reads for each species were trimmed using Trimmomatic v0.33 [4] and mapped to appropriate references (supplementary table 1) using BWA mem v0.7.17 (minimum and maximum insert sizes of 50 bp and 1000 bp respectively) [5]. Single nucleotide polymorphisms (SNPs) were called using SAMtools v1.2 mpileup and BCFtools v1.2 (minimum base call quality of 50 and minimum root squared mapping quality of 30) as previously described [6]. Samples with reads that mapped to < 80% of the reference were excluded (n = 70). Variant sites were extracted from the resulting alignments using snp-sites v2.5.1 [7]. Whole species maximum likelihood phylogenetic trees were built using IQ-tree v1.6.5 accounting for constant sites (-fconst; determined using snp-sites -C) with the built-in model testing (-m MFP) to determine the best phylogenetic model and 1000 ultrafast bootstraps (-bb 1000) [8].

For higher-resolution phylogenies within fastBAPS lineages, recombinant regions were identified and removed from alignments using GUBBINS [9] and new phylogenetic trees were constructed as described above. Pairwise SNP distances were calculated for all pairs of isolates using pairsnp [10].

Global collections

To provide context for each the isolates sequenced for each species in this study, datasets consisting of published sequenced isolates were assembled (Supplementary File 3) [11–31]. Sequence data were downloaded from the European Nucleotide Archive (ENA) and trimmed;

Sample QC, mapping and phylogenetic tree construction were performed as detailed above. Only the first isolate from each patient was included from the RBH isolates for each species/subspecies.

Genome assemblies

A previously published pipeline was used to produce annotated assemblies [32]. Briefly, sequence reads were assembled with spades v 3.10.10 [33] and assemblies were improved by first scaffolding the assembled contigs using SSPACE v2.0 [34] and filling the sequence gaps with GapFiller v1.11 [35].

Transmission and epidemiological linkage

Genomic lineages were identified using fastBAPS [36] and new alignments were created for lineages ≥ ten isolates by aligning sequence reads for included isolates against the assembly that had the smallest number of contigs (using the method described above). In order to calculate a pairwise SNP threshold to determine putative transmission clusters within each genomic lineage, pairwise SNP distances for all isolates for each species in the RBH datasets were calculated. Using a previously described method [37], the transmission threshold for each species, regardless of lineage, was calculated by taking the 95^{th} percentile of the maximum within-patient isolate pairwise SNP distances for all patients and adding twice the number of mutations expected to occur in a six month period. To account for excess within-patient diversity observed in the *M. chimaera* FB1 and *M. avium* subsp. *avium* FB14 lineages (Fig A in S1 Data), pairwise SNP distances greater than 25 and 50 (assumed to result from infection with multiple lineages) were removed respectively before the above calculations were performed. Based on these results, the R library iGRAPH [38,39] and pairwise SNP thresholds of 16 (*M. intracellulare* and *M. avium* subsp. *hominissuis*), 30 (*M. chimaera*) and

58 SNPs (*M. avium* subsp. *avium*) were used to calculate putative transmission clusters in each genomic lineage. Finally, in order to identify possible epidemiological links between patients infected within the same transmission clusters, hospital stay records were examined for epidemiological contacts. The latter were defined as patients attending the same ward on the same day up to one year prior to the collection of the first sequenced isolate.

References

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Supplementary Table 1. Reference genomes used in this study.

Species/ subspecies	Reference	Length (Mb)	Country of isolation	Year(s) of isolation
M. intracellulare	ATCC 13950	5.4	South Korea	2012
M. avium subsp. *avium*	104	5.5	USA	1983
M. avium subsp. *hominissuis*	TH135	5.0	Japan	2004-2008
M. chimaera	DSM 44623	5.9	Italy	1999-2003

Supplementary Table 2. *Mycobacterium intracellulare* transmission clusters

fastBAPS cluster	Transmission cluster	Patients (n)	Bronchiectasis (n)	Cystic Fibrosis (n)	Other lung conditions (n)	No pre-existing lung condition (n)	Patients undergoing treatment (n)
Mi_FB2	Mi_FB2_1	2	0	2	0	0	1
Mi_FB3	Mi_FB3_1	16	8	7	1	0	4
Mi_FB5	Mi_FB5_1	3	2	0	1	0	2

Supplementary Table 3. *M. avium* subsp. *avium* transmission clusters

fastBAPS cluster	Transmission cluster	Patients (n)	Bronchiectasis (n)	Cystic Fibrosis (n)	Other lung conditio	No pre-existing lung	Patients undergoing

					ns (n)	conditio n (n)	treatment (n)
MAA_FB 5	MAA_FB5_ 1	10	6	2	2	0	4
MAA_FB 5	MAA_FB5_ 4	2	1	1	0	0	0
MAA_FB 5	MAA_FB5_ 5	2	1	0	1	0	1
MAA_FB 5	MAA_FB5_ 12	2	1	1	0	0	1
MAA_FB 7	MAA_FB7_ 1	2	2	0	0	0	1
MAA_FB 10	MAA_FB10 _1	2	0	2	0	0	0
MAA_FB 14	MAA_FB14 _1	2	2	0	0	0	1

Supplementary Table 4. *M. avium* subsp. *hominissuis* transmission clusters

fastBAPS cluster	Transmissio n cluster	Patien ts (n)	Bronchiecta sis (n)	Cystic Fibros is (n)	Other lung conditio ns (n)	No pre-existing lung conditio n (n)	Patients undergoi ng treatment (n)
MAH_FB	MAH_FB8_	16	7	4	3	1	0

8	1						
MAH_FB 10	MAH_FB10 _1	4	2	2	0	0	1
MAH_FB 11	MAH_FB11 _1	6	3	1	2	0	0
MAH_FB 12	MAH_FB12 _2	4	1	2	1	0	2
MAH_FB 14	MAH_FB14 _3	4	2	2	0	0	0
MAH_FB 14	MAH_FB14 _7	2	0	2	0	0	0
MAH_FB 14	MAH_FB14 _9	7	3	2	1	0	1

Supplementary Table 5. *Mycobacterium chimaera* transmission clusters

fastBAPS cluster	Transmission cluster	Patients (n)	Bronchiectasis (n)	Cystic Fibrosis (n)	Other lung conditions (n)	No pre-existing lung condition (n)	Patients undergoing treatment (n)
Mc_FB3	Mc_FB3_1	106	43	24	25	3	15
Mc_FB3	Mc_FB3_3	2	1	1	0	0	0
Mc_FB3	Mc_FB3_4	2	2	0	0	0	0
Mc_FB3	Mc_FB3_6	2	1	0	1	0	0
Mc_FB3	Mc_FB3_1	4	1	1	1	1	1

	3						
Mc_FB3	Mc_FB3_16	2	0	0	1	1	1
Mc_FB3	Mc_FB3_17	2	1	1	0	0	0
Mc_FB3	Mc_FB3_19	2	0	1	0	1	0
Mc_FB3	Mc_FB3_25	2	1	1	0	0	1
Mc_FB3	Mc_FB3_30	4	0	1	0	0	0
Mc_FB4	Mc_FB4_1	2	1	1	0	0	1
Mc_FB4	Mc_FB4_2	9	3	3	3	0	2
Mc_FB4	Mc_FB4_3	6	1	2	2	0	1

Supplementary Table 6. *Mycobacterium chimaera* epidemiological links (n =15 patients)

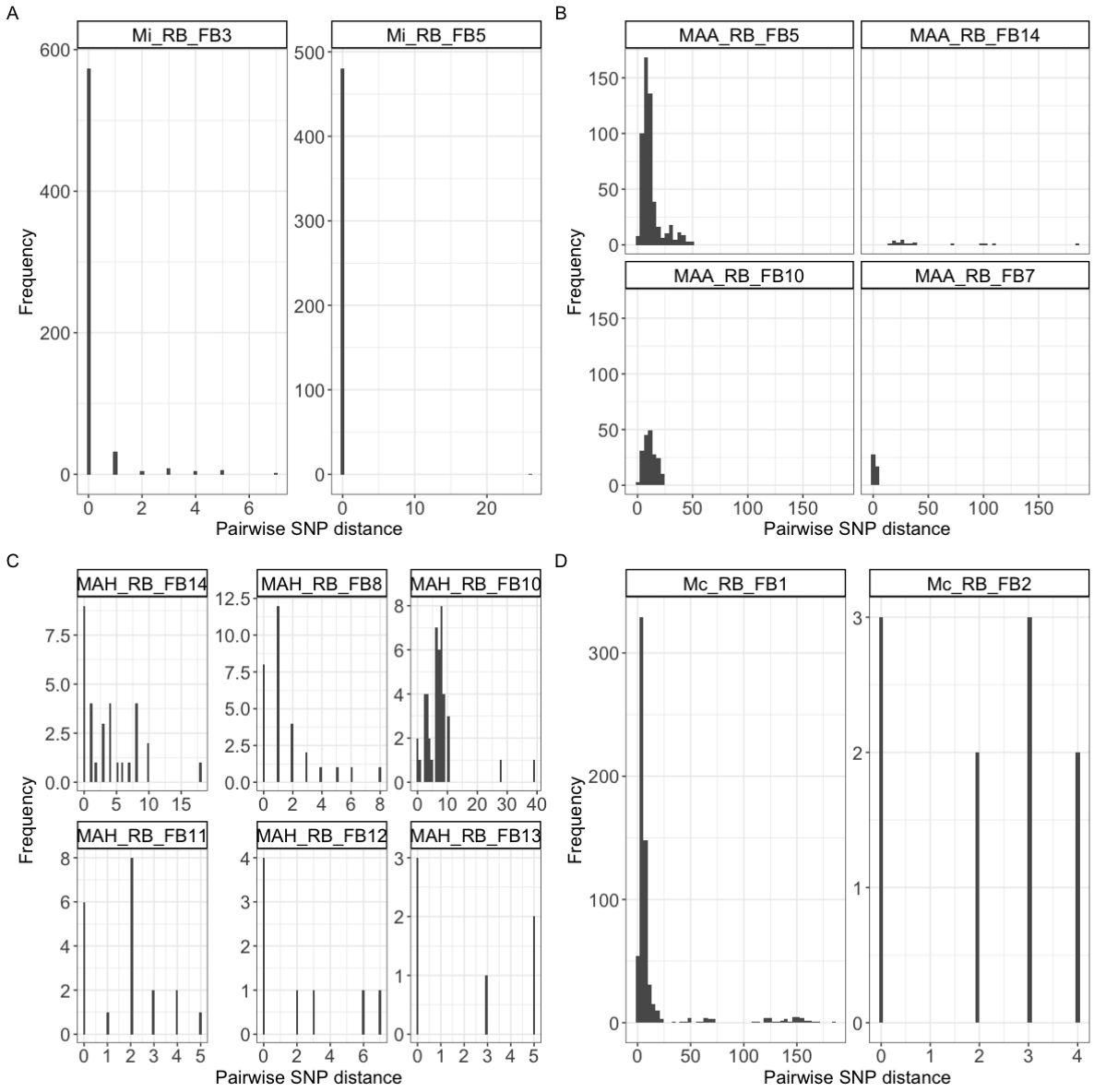
Patients with epidemiological link	Hospital ward	Dates of overlapping stay
24/218	FOUL	02/02/12-12/02/12
163/218	FOUL	13/02/12
175/218	FOUL	05/04/12-16/04/12
130/218	LIND	30/04/12
76/218	FOUL	06/06/12-11/06/12
32/163	LIND	29/06/12

79/241	LIND	05/07/12
241/272	LIND	06/07/12
177/186	LIND	06/08/12
122/175/306	FOUL	25/10/12-30/10/12
30/122	FOUL	05/11/12-06/11/12
30/218	FOUL	12/11/12-13/11/12

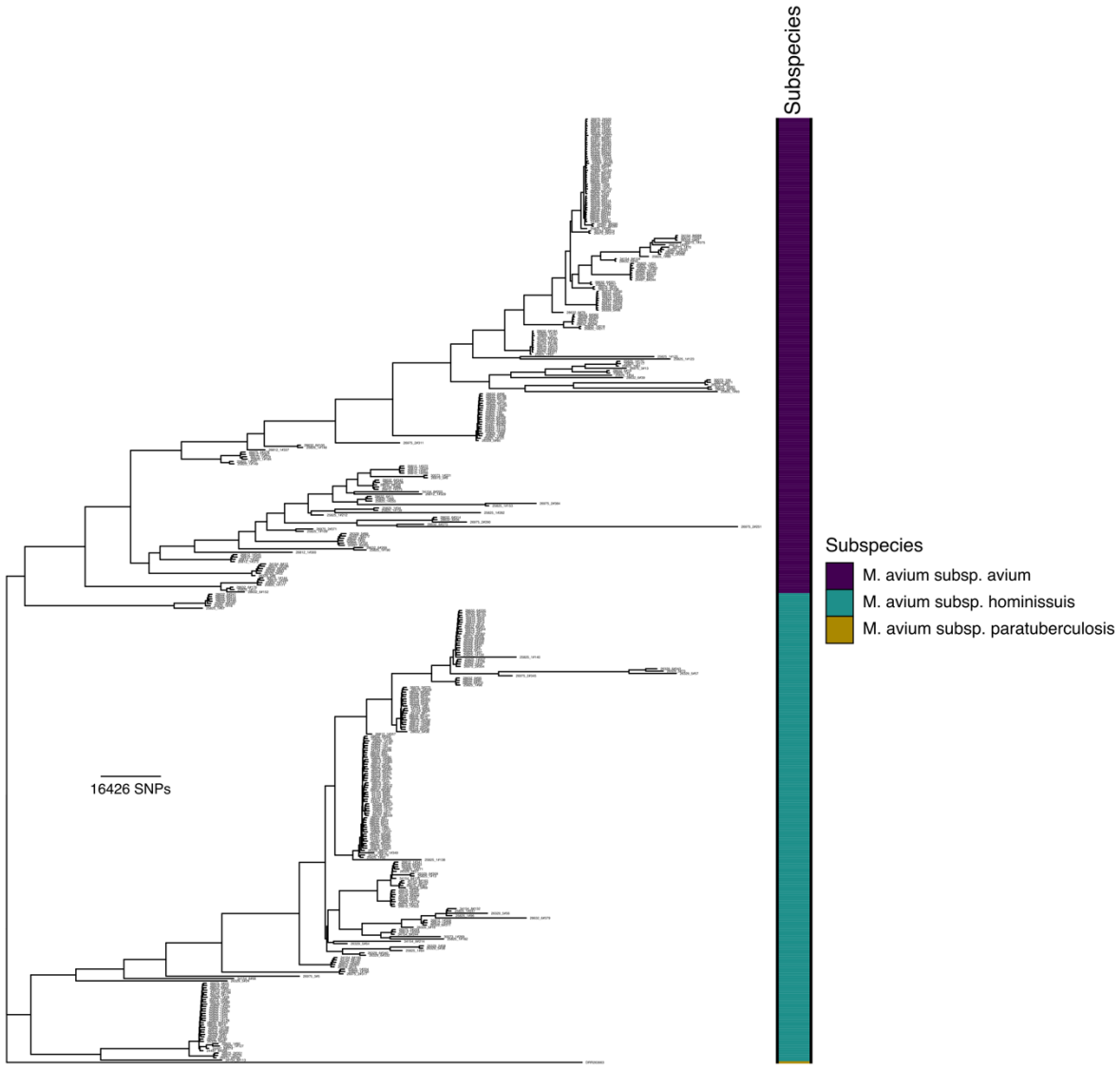
Supplementary Table 7. *Mycobacterium chimaera* global transmission clusters containing RBH isolates

fastBAPS cluster	Transmission cluster	Total isolates (n)	Patients (n)	HCU (n)	Other (n)
FB5	FB5_1	21	4	15	2
FB5	FB5_2	7	7	0	0
FB5	FB5_3	5	5	0	0
FB5	FB5_6	4	4	0	0
FB6_FB1	FB6_FB1_1	489	230	258	1
FB6_FB1	FB6_FB1_2	6	6	0	0
FB6_FB1	FB6_FB1_8	5	5	0	0
FB6_FB1	FB6_FB1_21	4	4	0	0
FB6_FB2	FB6_FB2_1	22	22	0	0
FB6_FB2	FB6_FB2_2	36	36	0	0
FB6_FB2	FB6_FB2_8	16	16	0	0
FB6_FB2	FB6_FB2_17	10	10	0	0

FB6_FB2	FB6_FB2_32	11	11	0	0
FB6_FB2	FB6_FB2_35	2	2	0	0
FB6_FB4	FB6_FB4_8	4	4	0	0
FB6_FB4	FB6_FB4_11	3	3	0	0
FB6_FB4	FB6_FB4_16	2	2	0	0

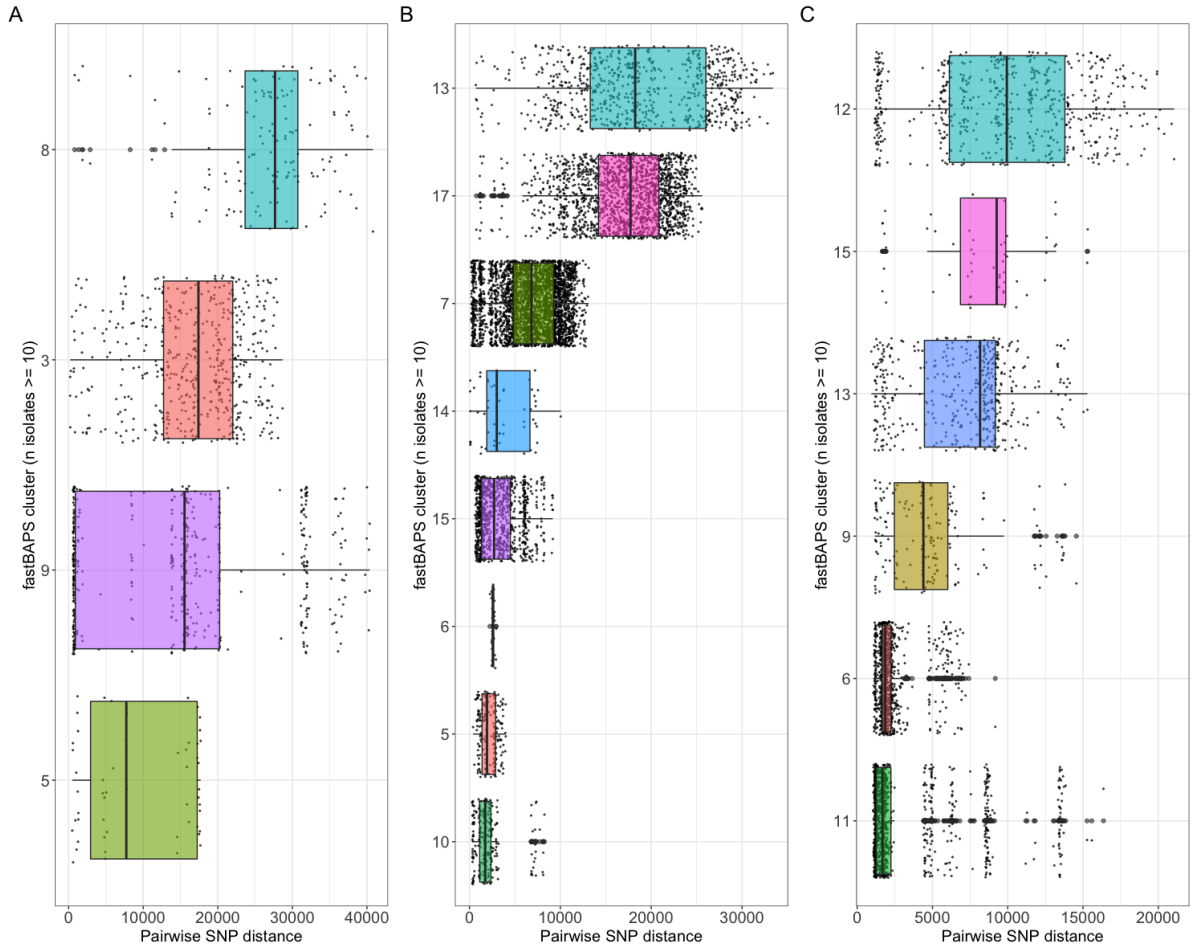


Supplementary Figure 1. Within-host isolate pairwise SNP diversity in fastBAPS lineages for A) *Mycobacterium intracellulare*; B) *Mycobacterium avium* subsp. *avium;* C) *Mycobacterium avium* subsp. *hominissuis* and D) *Mycobacterium chimaera*.

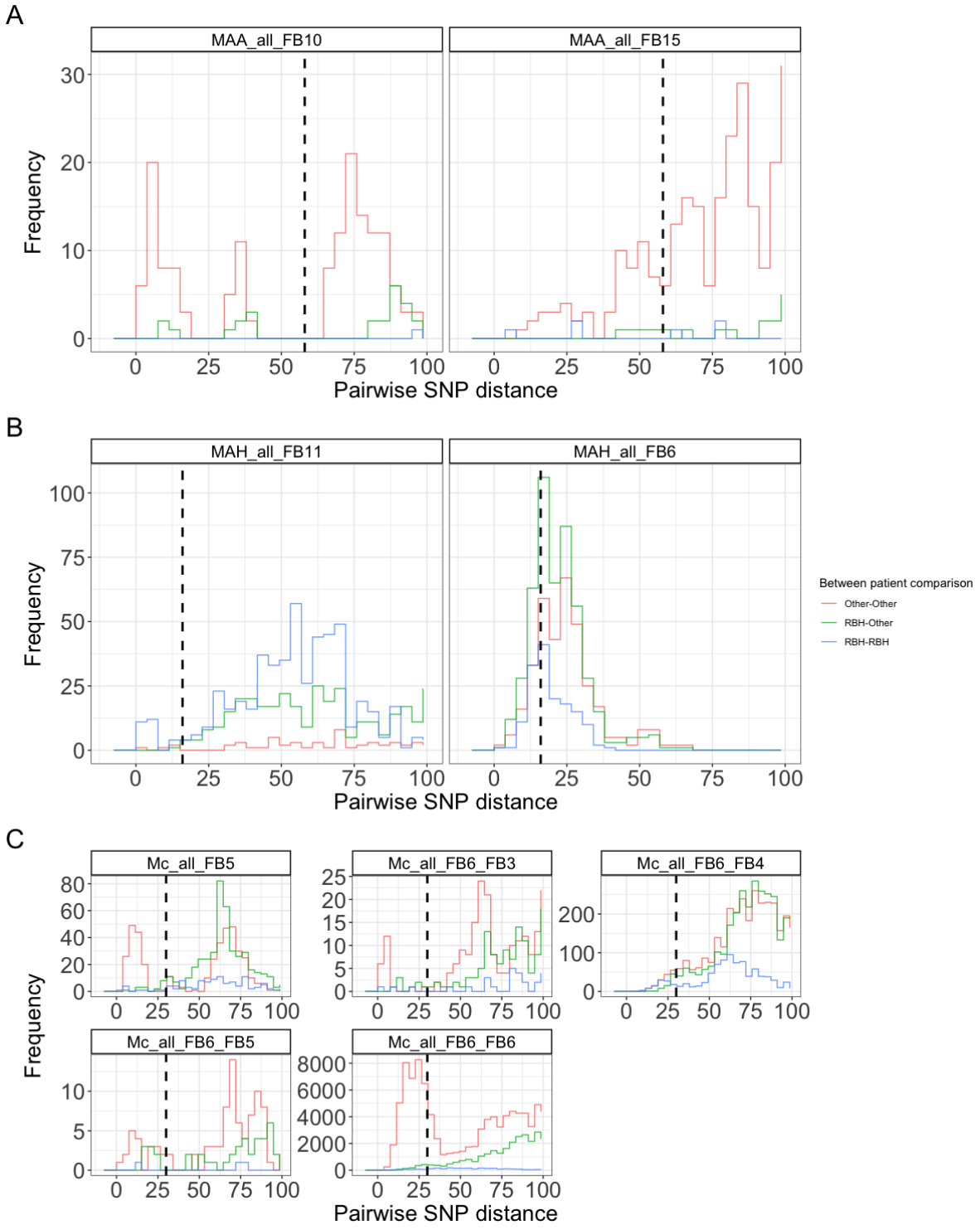


Supplementary Figure 2. Population structure of *Mycobacterium avium* at the Royal Brompton Hospital. Maximum likelihood phylogenetic tree of 406 *Mycobacterium avium* isolates rooted with a *Mycobacterium avium* subsp. *paratuberculosis* isolate (DRR263663).

The subspecies of each isolate is shown in the datastrip to the right of the phylogeny. The scale bar is shown in SNPs per site.



Supplementary Figure 3. Global fastBAPS cluster pairwise SNP distance distributions. Boxplots showing isolate pairwise SNP distances for A) *Mycobacterium intracellulare*; B) *Mycobacterium avium* subsp. *avium* and C) *Mycobacterium avium* subsp. *hominissuis*



Supplementary Figure 4: Distribution of pairwise SNP distances between isolates from the Royal Brompton Hospital and other studies. Histograms showing distribution of

pairwise SNP distances for fastBAPS clusters for A) *Mycobacterium avium* subsp. *avium*; B) *Mycobacterium avium* subsp. *hominissuis* and C) *Mycobacterium chimaera*.