Table S1. Genomes of the *Mycobacterium abscessus* species used in this study.

Strain	**Accession No.**	**No. Protein**	**Reference**	**Subsp. by *rpoB***	**Subsp. by *hsp65***
M18	NZ_AJSC00000000.1	4,726	Ngeow et al. J. Bacteriol. 2012;194:4125	*M. massiliense*	*M. massiliense*
M115	NZ_AJLZ00000000.1	4,822	Ngeow et al. J. Bacteriol. 2012;194:4786	*M. massiliense*	*M. massiliense*
M154	NZ_AJMA00000000.1	4,601	Choo et al. J. Bacteriol. 2012; 194:4778)	*M. massiliense*	*M. massiliense*
M172	NZ_AJSE00000000.1	5,069	Choo et al. J. Bacteriol. 2012; 194:5128	*M. massiliense*	*M. massiliense*
M159	NZ_AJSD00000000.1	4,791	Ngeow et al. Genome Announc. 2013;1:e00669-13	*M. massiliense*	*M. massiliense*
M93	NZ_AJGF00000000.1	4,848	Choo et al. J. Bacteriol. 2012; 194:3278	*M. abscessus*	*M. abscessus*
M94	NZ_AJGG00000000.1	5,020	Choo et al. J. Bacteriol. 2012; 194:5724	*M. abscessus*	*M. abscessus*
M152	NZ_AKVT00000000.1	4,741	Ngeow et al. J. Bacteriol. 2012,194:6662	*M. abscessus*	*M. abscessus*
M156	NZ_AKVU00000000.1	4,919	Choo et al. Genome Announc. 2013;1: e00063-12	*M. massiliense*	*M. massiliense*
M139	NZ_AKVR00000000.1	4,876	Ngeow et al. J. Bacteriol. 2012;194:6002-6003	*M. massiliense*	*M. massiliense*
M24	AJLY00000000.2	5,336	Wong et al. J. Bacteriol. 2012;194:4457	*M. bolletii*	*M. bolletii*
M148	NZ_AKVV00000000.1	5,427	-	*M. massiliense*	*M. massiliense*
1S-152-0914	NZ_AKUJ00000000.1	4,718	-	*M. massiliense*	*M. massiliense*
1S-153-0915	NZ_AKUK00000000.1	4,725	-	*M. massiliense*	*M. massiliense*
1S-154-0310	NZ_AKUL00000000.1	4,720	-	*M. massiliense*	*M. massiliense*
2B-0107	NZ_AKUN00000000.1	4,615	-	*M. massiliense*	*M. massiliense*
2B-0307	NZ_AKUU00000000.1	4,617	-	*M. massiliense*	*M. massiliense*
2B-0626	NZ_AKUM00000000.1	4,609	-	*M. massiliense*	*M. massiliense*
2B-0912-R	NZ_AKUV00000000.1	4,616	-	*M. massiliense*	*M. massiliense*
2B-0912-S	NZ_AKUW00000000.1	4,615	-	*M. massiliense*	*M. massiliense*
2B-1231	NZ_AKUO00000000.1	4,615	-	*M. massiliense*	*M. massiliense*
3A0119R	NZ_AKUX00000000.1	5,182	-	*M. abscessus*	*M. abscessus*
3A0122R	NZ_AKUY00000000.1	5,126	-	*M. abscessus*	*M. abscessus*
3A0122S	NZ_AKUZ00000000.1	5,109	-	*M. abscessus*	*M. abscessus*
3A0731	NZ_AKVA00000000.1	5,268	-	*M. abscessus*	*M. abscessus*
3A0810R	NZ_AKUP00000000.1	5,192	-	*M. abscessus*	*M. abscessus*

3A0930R	NZ_AKVB00000000.1	5,173	-	*M. abscessus*	*M. abscessus*
3A0930S	NZ_AKVC00000000.1	5,149	--	*M. abscessus*	*M. abscessus*
4S0116R	NZ_AKVD00000000.1	4,661	-	*M. abscessus*	*M. abscessus*
4S0116S	NZ_AKVE00000000.1	4,661	-	*M. abscessus*	*M. abscessus*
4S0206	NZ_AKUT00000000.1	4,674	-	*M. abscessus*	*M. abscessus*
4S0303	NZ_AKTU00000000.1	4,668	-	*M. abscessus*	*M. abscessus*
4S0726RA	NZ_AKTV00000000.1	4,662	-	*M. abscessus*	*M. abscessus*
4S0726RB	NZ_AKTW00000000.1	4,668	-	*M. abscessus*	*M. abscessus*
5S-0421	NZ_AKTY00000000.1	5,064	-	*M. massiliense*	*M. massiliense*
5S-0422	NZ_AKTZ00000000.1	5,153	-	*M. massiliense*	*M. massiliense*
5S-0708	NZ_AKUA00000000.1	5,070	-	*M. massiliense*	*M. massiliense*
5S-0817	NZ_AKUB00000000.1	5,071	-	*M. massiliense*	*M. massiliense*
5S-0921	NZ_AKUQ00000000.1	5,078	-	*M. massiliense*	*M. massiliense*
5S-1212	NZ_AKUC00000000.1	5,064	-	*M. massiliense*	*M. massiliense*
5S-1215	NZ_AKUD00000000.1	5,028	-	*M. massiliense*	*M. massiliense*
5S0304	NZ_AKTX00000000.1	5,071	-	*M. massiliense*	*M. massiliense*
6G0125R	NZ_AKUE00000000.1	4,958	-	*M. abscessus*	*M. abscessus*
6G0125S	NZ_AKUF00000000.1	5,190	-	*M. abscessus*	*M. abscessus*
6G0212	NZ_AKUR00000000.1	4,971	-	*M. abscessus*	*M. abscessus*
6G0728R	NZ_AKUS00000000.1	5,200	-	*M. abscessus*	*M. abscessus*
6G0728S	NZ_AKUG00000000.1	5,196	-	*M. abscessus*	*M. abscessus*
6G1108	NZ_AKUH00000000.1	5,202	-	*M. abscessus*	*M. abscessus*
BD^T	NZ_AHAS00000000.1	4,849	Choi et al. J. Bacteriol. 2012; 194:2756-2757	*M. bolletii*	*M. bolletii*
Asan 50594	NC_021282.1	4,910	Kim et al. Genome Annouc. 2013;1: e00429-13	*M. massiliense*	*M. massiliense*
CCUG 48898^T	NZ_AKVF00000000.1	4,961	Tettelin et al. J. Bacteriol. 2012;194:5450	*M. massiliense*	*M. massiliense*

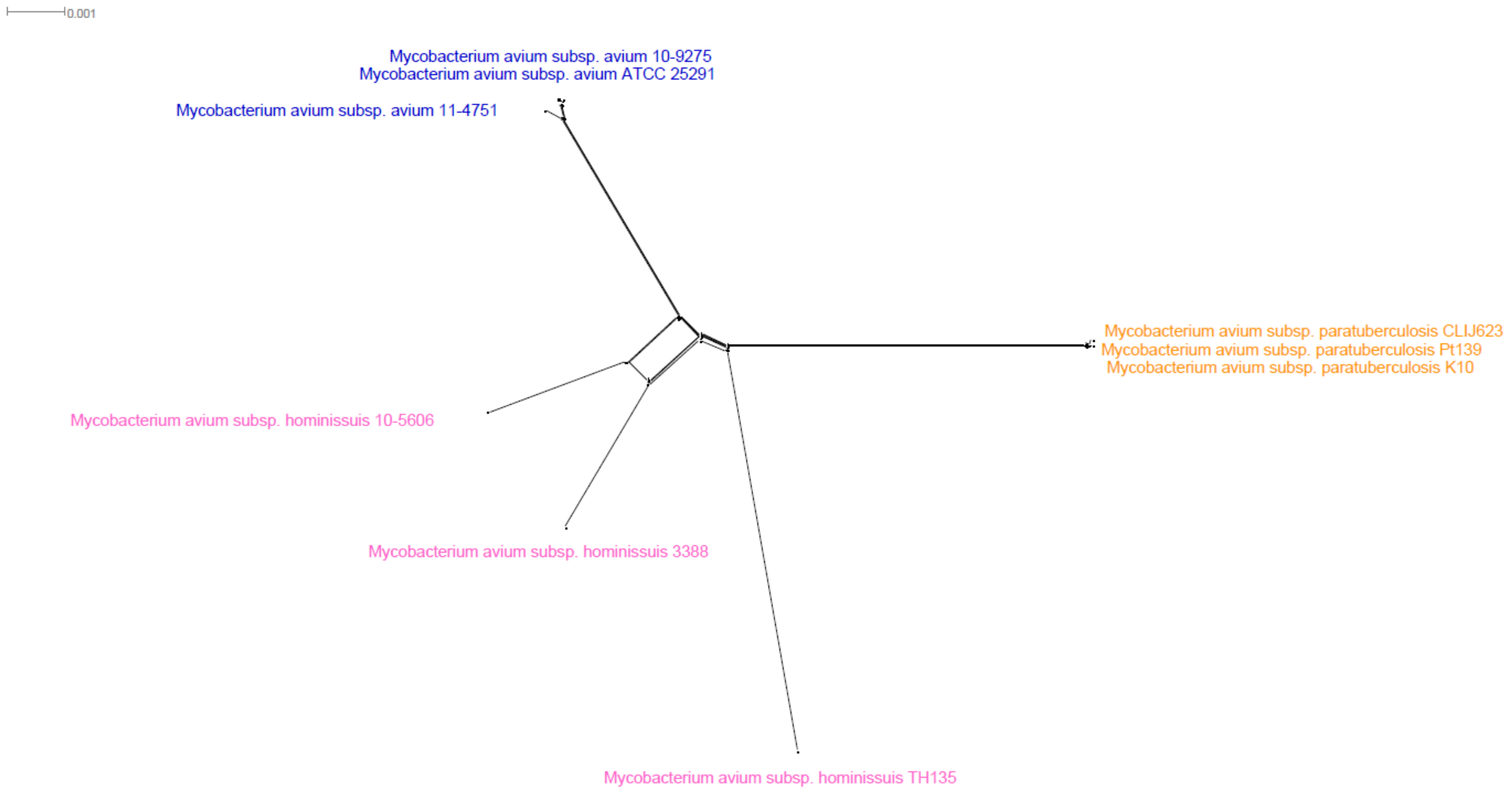

Figure S1. Neighbor-Net inferred tree for *M. avium* subsp. *paratuberculosis* (orange), *M. avium* subsp. *avium* (blue) and *M. avium* subsp. *hominissuis* (pink). The three established subspecies of *M. avium* complex are separated from each other in the phylogenomic tree based on single-copy orthologous sequences.

Table S2. Protein sequences and identified subspecies-specific amino acids

Gene	**Position**	***M. massiliense***	***M. abscessus***	***M. bolletii***
pseudouridine synthase	28	E	M	V
oxalate decarboxylase	168	L	P	T
putative regulatory protein TetR	22	D	Q	E
TetR family transcriptional regulator	104	D	A	T
hypothetical protein	68	N	K	D
putative short chain dehydrogenase/reductase	42	N	Q	K
hypothetical protein	114	A	M	V
hypothetical protein	291	A	V	I
hypothetical protein	122	V	I	M
magnesium-chelatase	323	D	E	G
hypothetical protein	155	V	L	A
	188	T	A	M
polyphosphate glucokinase PpgK	17	H	N	P
putative nucleotide-diphosphate-sugar epimerase	72	G	R	E
	225	H	R	G
HAD-superfamily hydrolase YfnB	169	H	Y	R
bifunctional pyrimidine operon regulatory protein/Uracil phosphoribosyltransferase	178	C	R	S
cinA-like protein	248	R	G	D
hypothetical protein	18	M	T	L
molybdenum ABC transporter permease	67	M	L	V
iron import ATP-binding/permease protein IrtA	256	P	A	S
	268	P	Q	R
hypothetical protein	194	K	T	M
hypothetical protein	7	T	A	V
hypothetical protein	11	V	I	A
hypothetical protein	26	V	I	T
	27	I	M	V
hypothetical protein	187	S	A	T
gamma-glutamyl phosphate reductase	196	I	V	A
putative monooxygenase	60	D	E	A
molybdopterin-guanine	68	Q	W	R

dinucleotide biosynthesis protein A				
putative drug antiporter protein	412	E	Q	R
hypothetical protein	182	V	L	I
TetR family transcriptional regulator	49	N	S	K
mycocerosate synthase	1172	V	T	A
	1220	G	V	E
hypothetical protein	12	A	V	M
hypothetical protein	37	L	V	M
hypothetical protein	53	T	V	A
dTDP-4-dehydrorhamnose reductase	40	T	G	S
hypothetical protein	13	R	P	A
mannose-6-phosphate isomerase ManA	66	I	T	A
	217	A	V	T
transcriptional regulator, TetR family	181	R	P	Q
hypothetical protein	197	A	G	S
hypothetical protein	381	S	K	R
2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase	136	L	V	A
peptidase C56 PfpI	68	R	T	A
dicarboxylic acid transport integral membrane protein KgtP	202	M	T	V
	231	T	A	V
	248	W	R	G
hypothetical protein	15	V	N	D
putative epimerase/dehydratase	105	I	V	T
	129	K	D	E
phosphomannomutase PmmB	99	Q	L	K