Supplementary Table

Table S1: Included sequences from this study and available public data.

Sample ID	Accession	Species	Origin	Country	Sequence source	Year of sampling	Patient ID
ntmscope_S_1	22006447	*M. simiae*	human	Germany	This study	2006	P1
ntmscope_S_2	22006448	*M. simiae*	human	Germany	This study	2007	P1
ntmscope_S_3	22006449	*M. simiae*	human	Germany	This study	2008	P1
ntmscope_S_5	22006450	*M. simiae*	human	Germany	This study	2010	P1
ntmscope_S_6	22006451	*M. simiae*	human	Germany	This study	2011	P1
ntmscope_S_7	22006452	*M. simiae*	human	Germany	This study	2011	P1
ntmscope_S_8	23002049	M. simiae	human	Germany	This study	2011	P2
ntmscope_S_9	23002050	M. simiae	human	Germany	This study	2011	P3
ntmscope_S_10	22006455	*M. simiae*	human	Germany	This study	2012	P1
ntmscope_S_11	23002051	M. simiae	human	Germany	This study	2012	P4
ntmscope_S_12	22006457	*M. simiae*	human	Germany	This study	2013	P1
ntmscope_S_14	22006459	*M. simiae*	human	Germany	This study	2014	P1
ntmscope_S_15	22006460	*M. simiae*	human	Germany	This study	2015	P1
ntmscope_S_16	22006461	*M. simiae*	human	Germany	This study	2015	P1
ntmscope_S_17	22006462	*M. simiae*	human	Germany	This study	2015	P5
ntmscope_S_18	22006463	*M. simiae*	human	Germany	This study	2015	P1
ntmscope_S_19	22006464	*M. simiae*	human	Germany	This study	2015	P1
ntmscope_S_20	23002052	M. simiae	human	Germany	This study	2015	P5
ntmscope_S_21	22006466	*M. simiae*	human	Germany	This study	2015	P6
ntmscope_S_22	23002053	M. simiae	human	Germany	This study	2016	P1
ntmscope_S_23	22006468	*M. simiae*	human	Germany	This study	2016	P6
ntmscope_S_24	22006469	*M. simiae*	human	Germany	This study	2016	P1
ntmscope_S_25	22006470	*M. simiae*	human	Germany	This study	2016	P5
ntmscope_S_26	22006471	*M. simiae*	human	Germany	This study	2016	P7
ntmscope_S_27	22006472	*M. simiae*	human	Germany	This study	2016	P5
ntmscope_S_28	22006473	*M. simiae*	human	Germany	This study	2017	P6
ntmscope_S_29	22006474	*M. simiae*	human	Germany	This study	2018	P1
ntmscope_S_30	22006475	*M. simiae*	human	Germany	This study	2018	P1
ntmscope_S_31	22006476	*M. simiae*	human	Germany	This study	2018	P1
ntmscope_S_33	22006478	*M. simiae*	human	Germany	This study	2018	P1
ntmscope_S_34	22006479	*M. simiae*	human	Germany	This study	2018	P1
ntmscope_S_35	22006480	*M. simiae*	human	Germany	This study	2019	P1
ntmscope_S_36	22006481	*M. simiae*	human	Germany	This study	2019	P1
ntmscope_S_39	23001063	M. interjectum	human	Germany	This study	2019	P11
ntmscope_S_40	23001064	M. interjectum	human	Germany	This study	2020	P12
ntmscope_S_41	23001065	M. palustre	human	Germany	This study	2021	P13
JCM 12377 / DSM 44165 / ATCC 25275	DRR161197	*M. simiae*	zoonotic	India	Public	NA	NA
MsiGto	GCA0020930751	*M. simiae*	human	Mexico	Public	NA	NA
JAL-560-SIM	GCA0083706451	*M. simiae*	human	India	Public	NA	NA
ATCC 51234	SRR1801752	*M. genavense*	human	Switzerland	Public	NA	NA
ATCC BAA-614	GCA0001641351	*M. parascrofulaceum*	human	USA	Public	NA	NA
JCM 17783 / DSM 45059	DRR161230	*M. stomatepiae*	zoonotic	UK	Public	NA	NA
AFP003 / JCM 18430	GCA9001762552	*M. ahvazicum*	human	Iran	Public	NA	NA

DSM 44602 / ATCC BAA-256 / CIP 108974	GCA0258232551	*M. montefiorense*	zoonotic	USA	Public	NA	NA
BS	GCA0031127751	*M. montefiorense*	zoonotic	Japan	Public	NA	NA
NJB14195	DRR357477	*M. montefiorense*	zoonotic	Japan	Public	NA	NA
JCM 32072 / DSM 46748 / UN-152	GCA0023563151	*M. shigaense*	human	Japan	Public	NA	NA
SCY	GCA0035156451	*M. shigaense*	human	China	Public	NA	NA
JCM 14742 / DSM 44553	DRR161215	*M. parmense*	human	Italy	Public	NA	NA
DSM 44572	GCA0258214851	*M. palustre*	environmental	Finland	Public	NA	NA
JCM 13016 / DSM 44616	DRR161205	*M. saskatchewanense*	human	Canada	Public	NA	NA
JCM 13573 / DSM 44627 / CIP 106428	DRR161209	*M. kubicae*	human	USA	Public	NA	NA
NJH_MKUB1	GCA0142633151	*M. kubicae*	human	USA	Public	NA	NA
NJH_MKUB2	GCA0142633351	*M. kubicae*	human	USA	Public	NA	NA
DSM 46749 / IEC26	GCA0258233851	*M. paraense*	human	Brazil	Public	NA	NA
FI-07156	GCA0021022951	*M. paraense*	human	Italy	Public	NA	NA
IEC33	GCA0021018251	*M. paraense*	human	Brazil	Public	NA	NA
FI-10043	GCA0021023051	*M. paraense*	human	Italy	Public	NA	NA
JCM 14749 / DSM 44852	DRR161214	*M. florentinum*	human	Italy	Public	NA	NA
JCM 14842 / DSM 44471	DRR161216	*M. heidelbergense*	human	Germany	Public	NA	NA
BAA-832 / DSM 45441	GCA0021023551	*M. sherrisii*	human	Germany	Public	NA	NA
BC1_M4	GCA0017223251	*M. sherrisii*	human	USA	Public	NA	NA
DSM 44049	GCA0258220651	*M. intermedium*	human	Germany	Public	NA	NA
HMC2_M5	GCA0017223451	*M. intermedium*	human	USA	Public	NA	NA
ATCC 51457T / DSM 44064	GCA0258214151	*M. interjectum*	human	Germany	Public	NA	NA
DSM 45397 / FI95228	GCA0021021551	*M. europaeum*	human	Italy	Public	NA	NA
CSUR P1344	GCA0013735151	*M. europaeum*	human	France	Public	NA	NA
ATCC 51985	SRR21133400	*M. lentiflavum*	human	Germany	Public	NA	NA
CSUR P1491	GCA0013733951	*M. lentiflavum*	human	France	Public	NA	NA
DSM 44626	GCA0021024151	*M. triplex*	human	USA	Public	NA	NA
	ERR6397219	*M. simiae*	human	Ethiopia	Public	NA	NA
24_Tigray_TB	ERR6397169	*M. simiae*	human	Ethiopia	Public	NA	NA
SAKAN MW_1	SRR12285193	*M. simiae*	human	India	Public	NA	NA
22b08e69-675c-4c13-8069-8eb1079be380	SRR6045477	*M. simiae*	human	UK	Public	NA	NA
SAMD00013969	DRR016026	*M. lentiflavum*	unknown	Japan	Public	NA	NA
SAMD00013336	DRR016032	*M. triplex*	unknown	Japan	Public	NA	NA
MMMOSAM:d904d2c1-3b0d-4c6c-90b1-5d8efa060272	SRR6045747	*M. lentiflavum*	human	United Kingdom	Public	2016	NA
SAMD00200838	DRR205177	*M. shigaense*	human	Japan	Public	2018	NA
SAMD00200837	DRR205178	*M. shigaense*	human	Japan	Public	2019	NA
myc97	SRR5052582	*M. genavense*	zoonotic	USA	Public	2005	NA
myc132	SRR5052583	*M. genavense*	zoonotic	USA	Public	2004	NA
myc88	SRR5052584	*M. genavense*	zoonotic	USA	Public	1997	NA
myc68	SRR5052586	*M. genavense*	zoonotic	USA	Public	2014	NA
myc95	SRR5052587	*M. genavense*	zoonotic	USA	Public	2004	NA
myc74	SRR5052589	*M. genavense*	zoonotic	USA	Public	2004	NA

myc108	SRR5052590	*M. genavense*	zoonotic	USA	Public	1998	NA
myc101	SRR5052591	*M. genavense*	zoonotic	USA	Public	2006	NA
myc73	SRR5052592	*M. genavense*	zoonotic	USA	Public	2004	NA
myc117	SRR5052594	*M. genavense*	zoonotic	USA	Public	2007	NA
myc75	SRR5052595	*M. genavense*	zoonotic	USA	Public	2004	NA
myc128	SRR5052596	*M. genavense*	zoonotic	USA	Public	2014	NA
myc130	SRR5052597	*M. genavense*	zoonotic	USA	Public	2010	NA
myc102	SRR5052599	*M. genavense*	zoonotic	USA	Public	2007	NA
myc105	SRR5052600	*M. genavense*	zoonotic	USA	Public	2004	NA
myc127	SRR5052602	*M. genavense*	zoonotic	USA	Public	2015	NA
myc115	SRR5052604	*M. genavense*	zoonotic	USA	Public	2004	NA
myc110	SRR5052606	*M. genavense*	zoonotic	USA	Public	2012	NA
myc93	SRR5052609	*M. genavense*	zoonotic	USA	Public	1996	NA
myc70	SRR5052612	*M. genavense*	zoonotic	USA	Public	2004	NA
myc72	SRR5052613	*M. genavense*	zoonotic	USA	Public	2006	NA
myc99	SRR5052614	*M. genavense*	zoonotic	USA	Public	2008	NA
myc91	SRR5052615	*M. genavense*	zoonotic	USA	Public	2008	NA
myc71	SRR5052617	*M. genavense*	zoonotic	USA	Public	2002	NA
myc122	SRR5052618	*M. genavense*	zoonotic	USA	Public	2012	NA
myc98	SRR5052619	*M. genavense*	zoonotic	USA	Public	2012	NA
myc83	SRR5052620	*M. genavense*	zoonotic	USA	Public	2011	NA
myc125	SRR5052621	*M. genavense*	zoonotic	USA	Public	2015	NA
myc103	SRR5052622	*M. genavense*	zoonotic	USA	Public	2015	NA
myc78	SRR5052623	*M. genavense*	zoonotic	USA	Public	2008	NA
myc79	SRR5052624	*M. genavense*	zoonotic	USA	Public	2010	NA
myc131	SRR5052627	*M. genavense*	zoonotic	USA	Public	2015	NA
myc129	SRR5052628	*M. genavense*	zoonotic	USA	Public	2014	NA
myc119	SRR5052629	*M. genavense*	zoonotic	USA	Public	2012	NA
myc82	SRR5052630	*M. genavense*	zoonotic	USA	Public	2011	NA
myc89	SRR5052631	*M. genavense*	zoonotic	USA	Public	2000	NA
myc106	SRR5052633	*M. genavense*	zoonotic	USA	Public	2015	NA
myc112	SRR5052634	*M. genavense*	zoonotic	USA	Public	1995	NA
myc114	SRR5052635	*M. genavense*	zoonotic	USA	Public	2015	NA
myc109	SRR5052636	*M. genavense*	zoonotic	USA	Public	2014	NA
myc107	SRR5052637	*M. genavense*	zoonotic	USA	Public	1996	NA
myc81	SRR5052640	*M. genavense*	zoonotic	USA	Public	2010	NA
myc100	SRR5052641	*M. genavense*	zoonotic	USA	Public	2007	NA
myc113	SRR5052643	*M. genavense*	zoonotic	USA	Public	2015	NA
myc47	SRR5043010	*M. genavense*	zoonotic	USA	Public	2014	NA
SP1876	SRR23562882	*M. saskatchewanense*	zoonotic	Italy	Public	2022	NA
CF00071-00221.MLENT	SRR7800693	*M. lentiflavum*	human	USA	Public	1905	NA
SAMD00162314	DRR171302	*M. interjectum*	unknown	Japan	Public	NA	NA

NA – not available

Table S2: Resistance, stress and virulence genes in 127 *M. simiae* complex isolates. *Some isolates had two acr3 genes located on different contigs. AMR-antimicrobial resistance.

AMR			
Gene	**antibiotic class**	**Isolates [n]**	**[%]**
aac(2')-Ic	AMINOGLYCOSIDE	121	95.3
aac(2')-Ie	AMINOGLYCOSIDE	2	1.6
abaF	FOSFOMYCIN	114	89.8
aph(3')-Ia	AMINOGLYCOSIDE	1	0.8
aph(3'')-Ib	AMINOGLYCOSIDE	1	0.8
aph(6)-Id	AMINOGLYCOSIDE	1	0.8
arr	RIFAMYCIN	109	85.8
blaC	BETA-LACTAM	3	2.4
blaE	BETA-LACTAM	1	0.8
blaF	BETA-LACTAM	34	26.8
blaLRA10	BETA-LACTAM	2	1.6
blaS	BETA-LACTAM	24	18.9
blaTEM	BETA-LACTAM	1	0.8
dfrA3	TRIMETHOPRIM	49	38.6
lmrC	FOSFOMYCIN	1	0.8
narA	IONOPHORE	4	3.1
rgt	RIFAMYCIN	114	89.8
STRESS			
Gene	**Type**	**Isolates [n]**	**[%]**
acr3	METAL	56 (89*)	44.1
arsA	METAL	2	1.6
arsC	METAL	126	99.2
arsN1	METAL	8	6.3
clpK	HEAT	2	1.6
golT	METAL	11	8.7
merC	METAL	4	3.1
qacE	BIOCIDE	1	0.8
trxLHR	HEAT	1	0.8
VIRULENCE			
Gene		**Isolates [n]**	**[%]**
bpsD		19	15.0
katP		127	100.0

Table S3: Specific cluster analysis of closely grouping isolates:

Cluster	Patients	Number of isolates	Positions used (%)	Median SNP distance	Range
Cluster 1	P1,P2,P3	25	5692254/5731089 (99.3 %)	1	0-5
Cluster 2	P5	4	5648931/5930572 (95.3 %)	2	0-2
Cluster 3	P4, P6	4	6242158/6315687 (98.8 %)	0	0

Supplementary figures

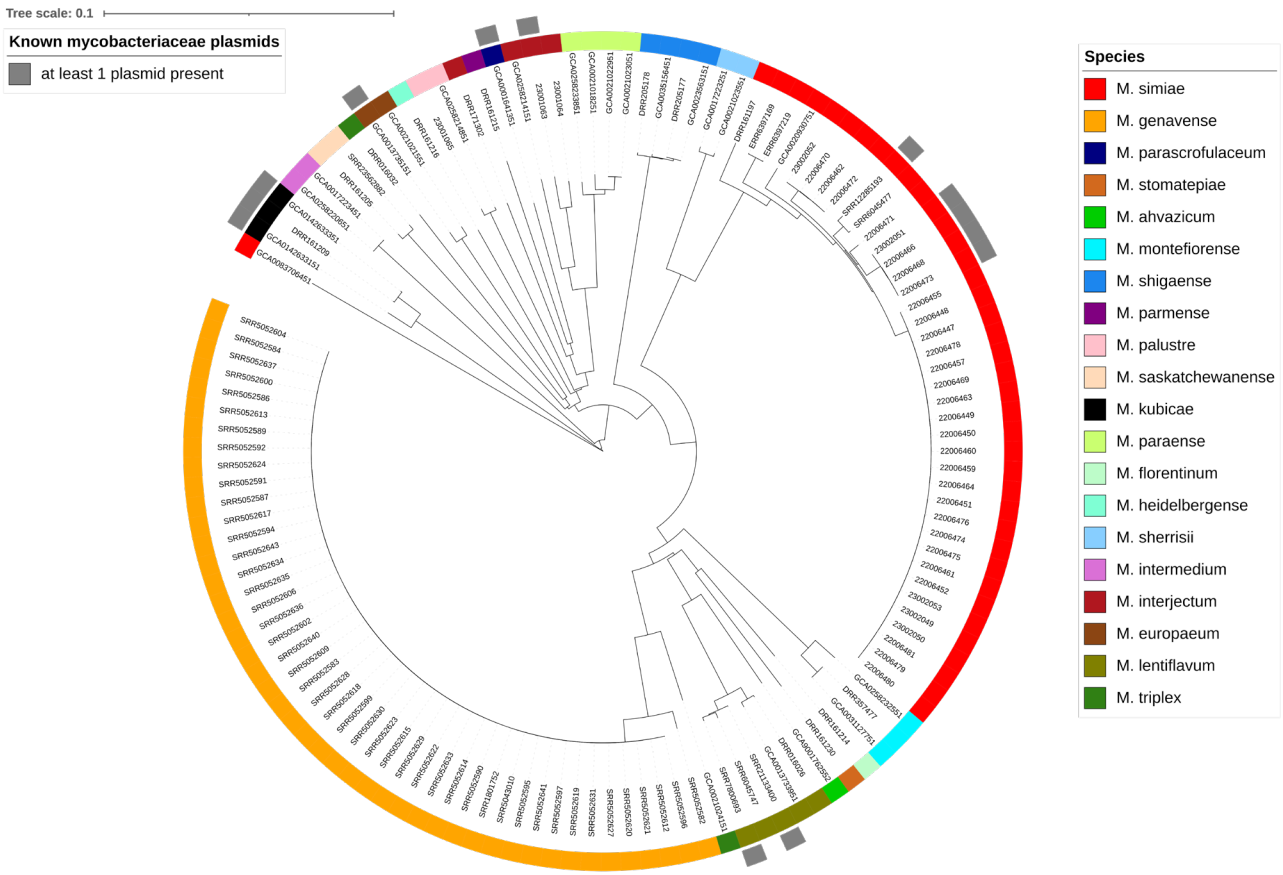

Figure S1: Presence of known mycobacteriaceae plasmids in *M. simiae* complex isolates.

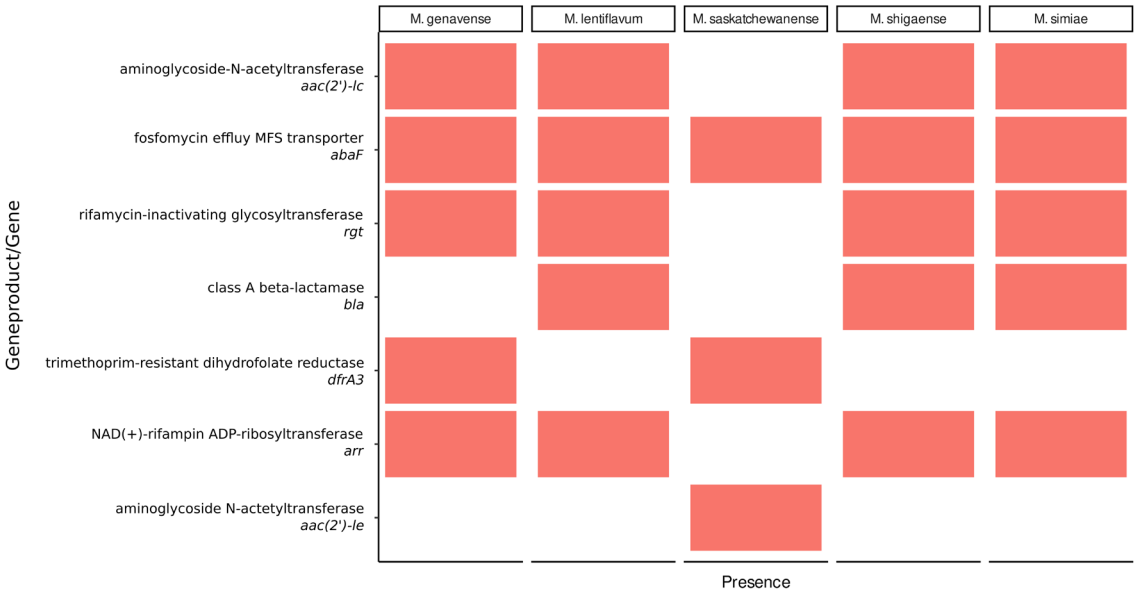

Figure S2: Presence of known antimicrobial resistance genes in different species of the *M. simiae* complex.

Figure S3: Timeline of included isolates, lung function, CT score after Song et al. and NTM therapy (detailed clinical information was only available from 2012 onwards). Colors represent the different patients according to the first plot panel. FEV1 – forced exspiratory volume in one second; CT – computed tomography; NA – not applicable.