Supplementary Tables:

Table S1: ENA accession codes for sequenced isolates in this study.

Isolate	Run accession	Experiment Accession	Sample Accession	Study accession
mabs1	ERR4298294	ERX4247020	ERS4787854	ERP015597
mabs1_2	ERR4298330	ERX4247056	ERS4787890	ERP015597
mabs2	ERR4298295	ERX4247021	ERS4787855	ERP015597
mabs3	ERR4298297	ERX4247023	ERS4787857	ERP015597
mabs3_2	ERR4298325	ERX4247051	ERS4787885	ERP015597
mabs4	ERR4298298	ERX4247024	ERS4787858	ERP015597
mabs4_2	ERR4298317	ERX4247043	ERS4787877	ERP015597
mabs5	ERR4298300	ERX4247026	ERS4787860	ERP015597
mabs5_2	ERR4298324	ERX4247050	ERS4787884	ERP015597
mabs6	ERR4298302	ERX4247028	ERS4787862	ERP015597
mabs7	ERR4298303	ERX4247029	ERS4787863	ERP015597
mabs7_2	ERR4298319	ERX4247045	ERS4787879	ERP015597
mabs8	ERR4298304	ERX4247030	ERS4787864	ERP015597
mabs9	ERR4298305	ERX4247031	ERS4787865	ERP015597
mabs10	ERR4298307	ERX4247033	ERS4787867	ERP015597
mabs10_2	ERR4298323	ERX4247049	ERS4787883	ERP015597
mabs11	ERR4298309	ERX4247035	ERS4787869	ERP015597
mabs12	ERR4298310	ERX4247036	ERS4787870	ERP015597
mabs13	ERR4298331	ERX4247057	ERS4787891	ERP015597
mabs14	ERR4298312	ERX4247038	ERS4787872	ERP015597
mabs15	ERR4298314	ERX4247040	ERS4787874	ERP015597
mabs16	ERR4298322	ERX4247048	ERS4787882	ERP015597
mabs16_2	ERR4298329	ERX4247055	ERS4787889	ERP015597
mabs17	ERR4298318	ERX4247044	ERS4787878	ERP015597
mabs17_2	ERR4298328	ERX4247054	ERS4787888	ERP015597
mbol1	ERR4298296	ERX4247022	ERS4787856	ERP015597
mbol2	ERR4298315	ERX4247041	ERS4787875	ERP015597
mmas1	ERR4298293	ERX4247019	ERS4787853	ERP015597
mmas1_2	ERR4298326	ERX4247052	ERS4787886	ERP015597
mmas2	ERR4298299	ERX4247025	ERS4787859	ERP015597
mmas3	ERR4298301	ERX4247027	ERS4787861	ERP015597
mmas3_2	ERR4298327	ERX4247053	ERS4787887	ERP015597
mmas4	ERR4298306	ERX4247032	ERS4787866	ERP015597
mmas5	ERR4298308	ERX4247034	ERS4787868	ERP015597
mmas6	ERR4298311	ERX4247037	ERS4787871	ERP015597
mmas7	ERR4298313	ERX4247039	ERS4787873	ERP015597
mmas8	ERR4298316	ERX4247042	ERS4787876	ERP015597
mmas9	ERR4298320	ERX4247046	ERS4787880	ERP015597
mmas10	ERR4298321	ERX4247047	ERS4787881	ERP015597
mabs: M. abscessus subsp. abscessus; mmas: M. abscessus subsp. massiliense;				
mbol: M. abscessus subsp. bolletii				

Table S2: Results of macrolide/aminoglycoside pDST and gDST (NTM-DR and WGS). Congruence of resistance prediction was 100%, while one isolate showed both WT and MUT reads of the *rrs* gene (*mabs12*).

Isolate	subspecies NTM-DR	*erm* (41) NTM-DR	*erm* (41) WGS	erm(41) WGS nucleotides	*rrl* -NTM-DR	*rrl* -WGS	pDSTM	*rrs* NTM DR	*rrs* WGS	pDSTA
mabs1	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs1_2	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs2	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs3	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs3_2	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs4	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs4_2	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs5	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs5_2	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs6	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs7	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs7_2	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs8	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs9	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs10	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs10_2	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs11	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs12	mabs	T28, functional	T28	522	WT	WT	resistant	MUT1	MUT1*	resistant
mabs13	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs14	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs15	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs16	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs16_2	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs17	mabs	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mabs17_2	mabs	T28, functional	T28	522	WT	WT	resistant	MUT1	MUT1	resistant
mbol1	mbol	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mbol2	mbol	T28, functional	T28	522	WT	WT	resistant	WT	WT	susceptible
mmas1	mmas	T28, truncated	T28	263	MUT4	MUT4	resistant	WT	WT	susceptible
mmas1_2	mmas	T28, truncated	T28	263	MUT4	MUT4	resistant	WT	WT	susceptible
mmas2	mmas	T28, truncated	T28	263	WT	WT	susceptible	WT	WT	susceptible
mmas3	mmas	T28, truncated	T28	263	WT	WT	susceptible	WT	WT	susceptible
mmas3_2	mmas	T28, truncated	T28	263	WT	WT	susceptible	WT	WT	susceptible
mmas4	mmas	T28, truncated	T28	263	WT	WT	susceptible	WT	WT	susceptible
mmas5	mmas	T28, truncated	T28	263	WT	WT	susceptible	WT	WT	susceptible
mmas6	mmas	T28, truncated	T28	260	WT	WT	susceptible	WT	WT	susceptible
mmas7	mmas	T28, truncated	T28	263	WT	WT	susceptible	WT	WT	susceptible
mmas8	mmas	T28, truncated	T28	260	WT	WT	susceptible	WT	WT	susceptible
mmas9	mmas	T28, truncated	T28	263	WT	WT	susceptible	WT	WT	susceptible
mmas10	mmas	T28, truncated	T28	263	WT	WT	susceptible	WT	WT	susceptible

rrl MUT4: A2059G; *rrs* MUT1: A1408G; WT: wild type; mabs: *M. abscessus* subsp. abscessus; *mmas*: *M. abscessus* subsp. *massiliense*; *mbol*: *M. abscessus* subsp. *bolletii*

pDSTM: phenotypic drug susceptibility testing of macrolides; pDSTA: phenotypic drug susceptibility testing of aminoglycosides

*48 reads WT, 24 reads MUT1

Table S3: Table showing a comparison between full phylogenetic analysis and cluster analysis for the corresponding clusters. In all clusters there was a much higher usage of the reference genome in the cluster analysis. In *mmas*, SNP difference and SNP ratio between both analyses were significantly larger than in *mabs*.

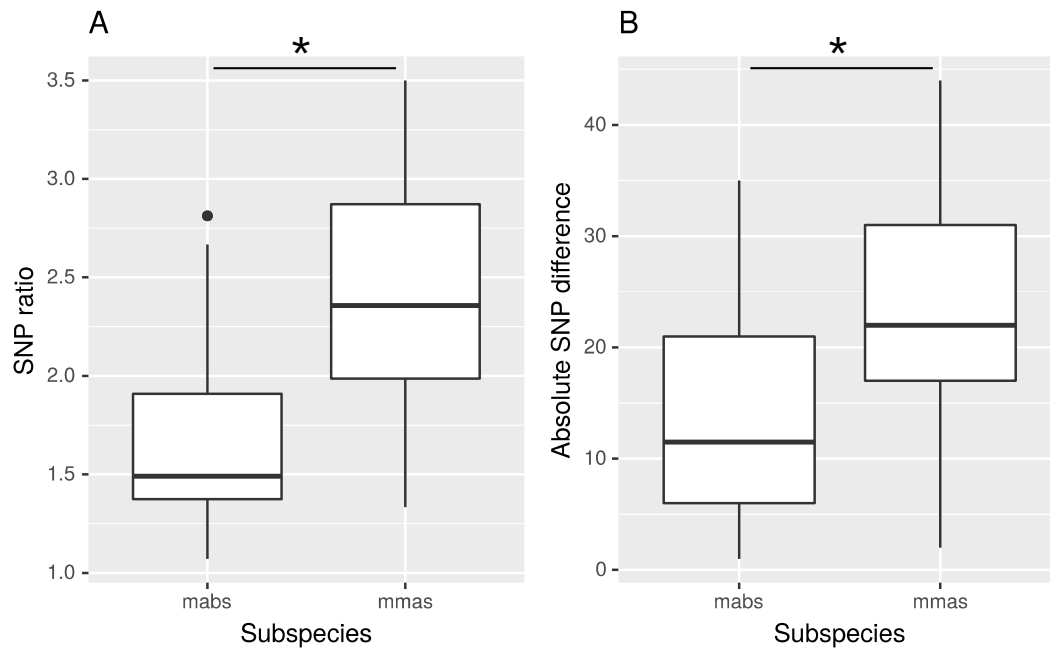| Analysed cluster	Size of reference genome		Usage of reference genome		Median SNP distance		Analyses comparison	
	full analysis	cluster analysis	full analysis	cluster analysis	full analysis	cluster analysis	SNP difference (median, range)	SNP-ratio (median)
M. *abscessus* subsp. *abscessus*								
mabs_d25_cluster1	5067172	5237165	88,57%	99,70%	10 (4-14)	11 (6-15)	1 (1-2)	1,22
mabs_d25_cluster2	5067172	5135369	88,57%	99,17%	21.5 (13-32)	28 (17-37)	5.5 (2-10)	1,32
mabs_d25_cluster3	5067172	5164744	88,57%	99,71%	24 (13-35)	42 (22-67)	19 (6-35)	1,7
M. *abscessus* subsp. *massiliense*								
mmas_d25_cluster1	5067172	5118935	85,53%	99,63%	14 (6-32)	33 (8-74)	22 (2-42)	2,33
mmas_d25_cluster2	5067172	4899047	85,53%	99,60%	11	15	4	1,36
mmas_d25_cluster3	5067172	4960781	85,53%	99,79%	17	55	38	3,23

Supplementary Figures:

Figure S1: SNP ratio and absolute SNP difference between full and cluster analyses in mabs and *mmas*. (A) SNP ratio was significantly larger in *mmas*, as was absolute SNP difference (B).