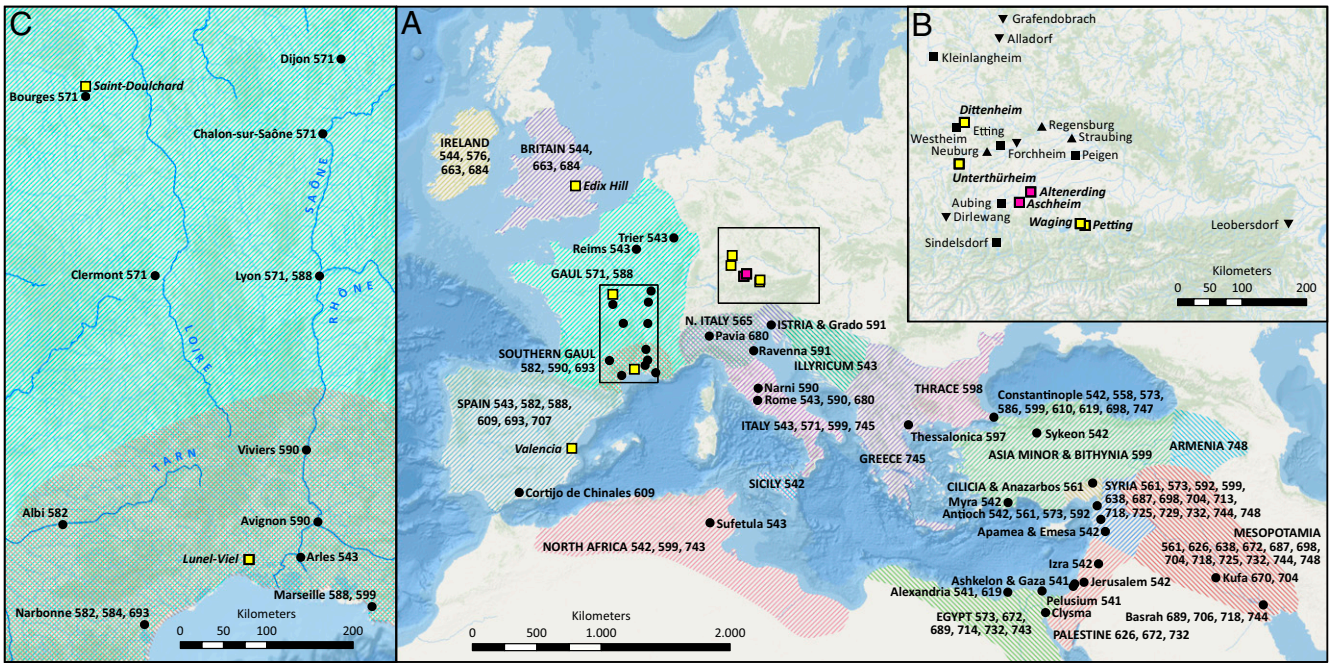

Fig. 1. Geographic extent of the First Pandemic and sampled sites. (*A*) Map of historically documented occurrences of plague (regions shaded, cities depicted by circles, both with respective years of occurrence) between 541 and 750 in Europe and the Mediterranean basin. All sources are given in *SI Appendix*. Sites with genomic evidence for *Y. pestis* are shown as pink (previously published) and yellow squares (presented here). (*B*) Enlarged rectangular space of *A* (*Right*) showing all sites in Germany and Austria that were included in this study. Sites tested negative are depicted in black upward-pointing triangles (burials dating before 541), squares (dating around 541–544), and downward-pointing triangles (dating after 544). (*C*) Enlarged *Inset* of *A* (*Left*) shows reported occurrences in France and main rivers.

On the pCD1 plasmid, one SNP was identified as missing in the Edix Hill genomes (EDI001.A, EDI003.A, EDI004.A), one as shared between both Saint-Doulchard genomes (LSD001.A, LSD023.A), and one as unique to the genome LSD001.A. One additional SNP was found on the pMT1 plasmid in the Valencia genome (VAL001.B). An analysis of the Aschheim genome as well as a SNP effect analysis is presented in *SI Appendix*, Tables S7 and S14.

SNPs shared by at least two genomes without a conflicting call in any other genome were evaluated as potentially shared SNPs among the First Pandemic lineage. We applied the exact same parameters as for the nonshared SNPs, but also considered positions with less than threefold coverage (*SI Appendix*, Table S11). Only SNPs that pass all three criteria of our SNP evaluation in at least half of the analyzed genomes (i.e., 6 out of 12) were accepted as true shared SNPs, reducing the number from 50 SNPs identified in a previous study (7)—after removal of nonshared and ambiguous SNPs—to 45.

The Waging sample (WAG001.A) had a genomic coverage too low for inclusion in our phylogenetic analysis. Since it was the only sample giving evidence for *Y. pestis* presence at this site, it was assessed for all SNPs that were either shared or unique in the other First Pandemic genomes. Visual inspection revealed 7 of the 43 shared SNPs to be present in the WAG001.A genome at low coverage (less than threefold) and one SNP absent in Edix Hill but potentially present in all other genomes. For both shared and unique SNPs, no conflicting positions were found. This strain could, therefore, be attributed to the First Pandemic lineage without, however, resolving its exact phylogenetic position (*SI Appendix*, Table S11).

Phylogenetic Analysis. A set of 233 modern *Y. pestis* genomes (*SI Appendix*, Table S12) as well as 7 Second Pandemic genomes, including a representative of the Black Death strain (London) and 7 post-Black Death genomes [14th-century Bolgar, 16th-century Ellwangen (12); 18th-century Marseille (13)], and an ancient genome from Tian Shan [DA101, second to third century (28)] were used for phylogenetic analyses alongside our First Pandemic genomes presented here (*SI Appendix*, Table S3) and the previously published genome of Altenerding. The *Y. pseudotuberculosis* isolate IP32953 (29) was used as an outgroup.

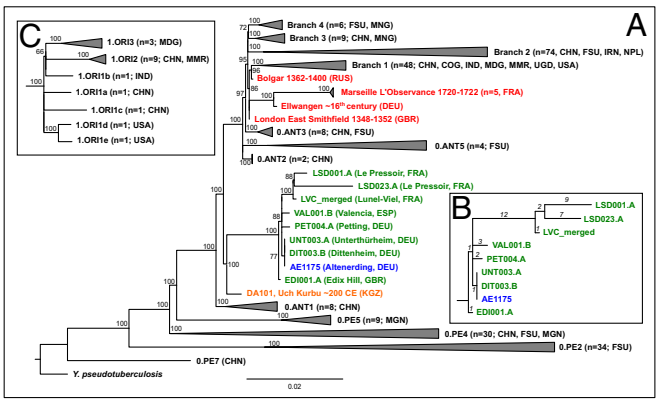

Fig. 2. Phylogenetic tree. (*A*) Maximum-likelihood tree with full SNP alignment (6,580 positions) of 233 modern *Y. pestis* and one *Y. pseudotuberculosis* genome, 10 published (second- to third-century Tian Shan in orange; Altenerding in blue; Second Pandemic in red) and eight genomes presented here (green) with country given in brackets (DEU = Germany, ESP = Spain, FRA = France, GBR = Great Britain, RUS = Russia). Numbers and origins of modern genomes are given in brackets (CHN = China, COG = Congo, FSU = Former Soviet Union, IND = India, IRN = Iran, MDG = Madagascar, MMR = Myanmar, MNG = Mongolia, NPL = Nepal, UGA = Uganda). Numbers on nodes are showing bootstrap values (1,000 iterations). (*B*) Detailed, manually drawn tree of the First Pandemic genomes showing all remaining SNP positions after SNP evaluation (number of SNPs given in italics). (*C*) Detailed tree of the 1.ORI clade within branch 1, showing the polytomy.