

## Description of Additional Supplementary Files:

**Supplementary Data 1:** Metadata for SRA sequencing runs associated with high *C. tetani* abundance predicted using the SRA taxonomy analysis tool.

**Supplementary Data 2:** Metadata and bioinformatic annotations for BioSamples containing ancient human DNA and *C. tetani*. Metadata includes SRA-derived information as well as newly added annotations based on analysis of acBins.

**Supplementary Data 3:** Identified archaeal and bacterial species in *C. tetani*-containing human aDNA samples. Total counts for each biosample are derived from their respective SRA taxonomic profiles.

**Supplementary Data 4:** Species identified in 38 BioSamples and their environmental associations based on known literature. All species listed were detected with greater than 2% abundance of the microbial community based on the SRA STAT results.

**Supplementary Data 5:** Estimated abundance (of microbial community) of environment-specific microbes in the bacterial and archaeal fraction of 38 BioSamples. Environment-specific microbes were defined as shown in Supplementary Data 4.

**Supplementary Data 6:** Kaiju taxonomic classification summary at the species level of assembled samples (contig count).

**Supplementary Data 7:** Statistics for assembled contigs assigned to *C. tetani* after blast-validation.

**Supplementary Data 8:** CheckM analysis of all 38 acBin BLAST-verified *C. tetani* contigs.

**Supplementary Data 9:** MapDamage analysis of acBin contig sets. Contigs were BLAST-validated as mapping to *C. tetani* and contigs encoding rRNAs and tRNAs were removed.

**Supplementary Data 10:** PyDamage analysis of acBin contig sets. Contigs were BLAST-validated as mapping to *C. tetani* and contigs encoding rRNAs and tRNAs were removed.

**Supplementary Data 11:** Pairwise average nucleotide identity values for a *C. tetani* bin from a human gut sample (SRR10479805) to acBins and modern genomes from related *Clostridium* species.

**Supplementary Data 12:** Pairwise average nucleotide identity values for *Clostridium* sp. clade X acBins and modern genomes from related *Clostridium* species.

**Supplementary Data 13:** Pairwise average nucleotide identity values for lineage Y acBin (GranCanaria008-Tooth) and modern genomes from related *Clostridium* species.

**Supplementary Data 14:** Orthogroups of all modern *C. tetani* genomes and all acBins. Any orthogroup without at least one protein on a blast-validated contig and/or without at least one full length protein has been removed.

**Supplementary Data 15:** *C. tetani* orthogroups that contain at least one *C. tetani* E88 plasmid protein and their frequency in the other genomes/acBins. Protein descriptions are listed for orthogroups with  $\geq 90\%$  frequency in high-completeness acBins.

**Supplementary Data 16:** *C. tetani* orthogroups that contain at least one *C. tetani* E88 sporulation-related protein and their frequency in the other genomes/acBins.

**Supplementary Data 17:** Per-sample coverage statistics and correlations for the *C. tetani* chromosome, plasmid, *repA*, *colT* gene, and *tent* gene.

**Supplementary Data 18:** Comparison of assembled *tent* sequences from ancient DNA samples with modern *tent* sequences.

**Supplementary Data 19:** Detected SNPs in *tent* sequences from acBins and modern *C. tetani* strains relative to *tent*/E88 reference.

**Supplementary Data 20:** *tent* variants detected in ancient samples using the Octopus variant caller. See VCF file format for header information.

**Supplementary Data 21:** Analysis of SNPs present in *tent* sequences from Chinchorro\_Mummy\_bone, El\_Yaral\_Bone, and Chibaya\_AltaBone.

**Supplementary Data 22:** FASTQ processing statistics for ancient DNA samples with high predicted *C. tetani* content.

**Supplementary Data 23:** Genomes of *C. tetani* strains downloaded from the NCBI database and associated metadata.