

Toxigenic *Corynebacterium diphtheriae* Infection in a Cat, Texas, USA

Appendix

Methods

C. diphtheriae PC1297 was grown on trypticase soy agar with 5% sheep blood at 37 °C for 24 h. Genomic DNA was extracted using the Maxwell RSC whole Blood DNA kit (Promega, <https://www.promega.com>) and the concentration was determined using the Qubit dsDNA broad range quantification kit (Thermo Fisher Scientific, <https://www.thermofisher.com>). Paired-end libraries (2 X 250bp) were prepared with the NEBNext Ultra DNA Library Prep Kit (New England Biolabs, <https://www.neb.com>) and sequenced on a MiSeq using reagent v2, 500-cycle kit (Illumina, <https://www.illumina.com>). Raw Illumina read quality was assessed using FastQC v0.11.5 (1) before trimming and filtering with BBduk (2) and Cutadapt (3). Trimmed reads were de novo assembled using SPAdes v3.9 (4) and evaluated with QUAST v4.5 (5).

Genome sequence-based molecular typing by multi-locus sequence typing (MLST) was determined from the assembled contigs according to stringMLST (6), and further confirmed with trimmed sequencing reads using SRST2 (7). The presence of diphtheria toxin homologs was determined by tBLASTn query of the assembled genomes using select references: Corynephage beta A and B subunits (NCBI accession no. P00588), Corynephage omega beta Diphtheria toxin (NCBI accession no. P00587), and Corynephage beta Diphtheria toxin homolog (NCBI accession no. P00589).

Publicly available *C. diphtheriae* genomic data for 273 isolates representing 270 unique sequence types were retrieved from the NCBI Sequence Read Archive (SRA) for phylogenetic comparison (Appendix Table 1). Raw reads were trimmed and filtered as described above. SNPs were determined by mapping all trimmed reads to the reference genome of NCTC13129 (NC_002935) using Snippy v4.3.8 with default settings (8). The phylogeny was reconstructed

from the resulting core SNP alignment using maximum-likelihood with RaxML v8.2.9 (9) and the tree was visualized with iTOL v6 (10) (Appendix Figure 1).

References

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Appendix Table. *C. diphtheriae* isolate sequences used for phylogenetic analysis*

| ID | atpA | dnaE | dnaK | fusA | leuA | odhA | rpoB | ST | Species (animal)† |
|------------|------|------|------|------|------|------|------|-----|-----------------------|
| ERR2094322 | 2 | 1 | 4 | 1 | 3 | 3 | 5 | 97 | <i>C. diphtheriae</i> |
| ERR2094324 | 2 | 26 | 55 | 29 | 3 | 3 | 2 | 184 | <i>C. diphtheriae</i> |
| ERR2094326 | 3 | 2 | 4 | 30 | 3 | 3 | 2 | 251 | <i>C. diphtheriae</i> |
| ERR2094331 | 28 | 1 | 20 | 19 | 3 | 16 | 20 | 102 | <i>C. diphtheriae</i> |
| ERR2094333 | 30 | 23 | 63 | 25 | 3 | 3 | 28 | 217 | <i>C. diphtheriae</i> |
| ERR2094334 | 4 | 11 | 13 | 7 | 3 | 56 | 27 | 432 | <i>C. diphtheriae</i> |
| ERR2094336 | 28 | 2 | 60 | 30 | 3 | 3 | 3 | 367 | <i>C. diphtheriae</i> |
| ERR2094337 | 2 | 1 | 20 | 19 | 42 | 16 | 31 | 412 | <i>C. diphtheriae</i> |
| ERR2167138 | 2 | 1 | 20 | 16 | 3 | 3 | 5 | 83 | <i>C. diphtheriae</i> |
| ERR2173761 | 4 | 1 | 102 | 4 | 5 | 5 | 35 | 503 | <i>C. diphtheriae</i> |
| ERR2173765 | 2 | 51 | 9 | 60 | 8 | 3 | 2 | 486 | <i>C. diphtheriae</i> |
| ERR2173766 | 30 | 1 | 93 | 25 | 3 | 3 | 6 | 398 | <i>C. diphtheriae</i> |
| ERR2173767 | 4 | 46 | 4 | 1 | 5 | 3 | 2 | 473 | <i>C. diphtheriae</i> |
| ERR2173768 | 22 | 4 | 60 | 62 | 3 | 1 | 4 | 505 | <i>C. diphtheriae</i> |
| ERR2173771 | 4 | 10 | 3 | 1 | 7 | 3 | 13 | 469 | <i>C. diphtheriae</i> |
| ERR2173772 | 3 | 53 | 32 | 19 | 3 | 4 | 4 | 506 | <i>C. diphtheriae</i> |
| ERR2173773 | 2 | 2 | 60 | 2 | 3 | 3 | 2 | 474 | <i>C. diphtheriae</i> |
| ERR2173774 | 30 | 10 | 102 | 4 | 3 | 3 | 35 | 502 | <i>C. diphtheriae</i> |
| ERR2217012 | 2 | 3 | 3 | 2 | 7 | 3 | 3 | 406 | <i>C. diphtheriae</i> |
| ERR2217013 | 2 | 2 | 9 | 25 | 3 | 3 | 2 | 302 | <i>C. diphtheriae</i> |
| ERR2217014 | 1 | 1 | 88 | 1 | 29 | 52 | 4 | 379 | <i>C. diphtheriae</i> |
| ERR2217016 | 4 | 21 | 29 | 1 | 3 | 3 | 3 | 120 | <i>C. diphtheriae</i> |
| ERR2217017 | 13 | 4 | 8 | 44 | 3 | 3 | 13 | 433 | <i>C. diphtheriae</i> |
| ERR3331350 | 16 | 4 | 8 | 1 | 2 | 30 | 9 | 151 | <i>C. diphtheriae</i> |
| ERR3331352 | 3 | 2 | 4 | 19 | 3 | 16 | 9 | 455 | <i>C. diphtheriae</i> |
| ERR3331354 | 5 | 2 | 7 | 1 | 3 | 5 | 8 | 10 | <i>C. diphtheriae</i> |
| ERR3331355 | 3 | 2 | 3 | 6 | 3 | 3 | 2 | 67 | <i>C. diphtheriae</i> |
| ERR3331356 | 16 | 8 | 50 | 1 | 2 | 30 | 9 | 161 | <i>C. diphtheriae</i> |
| ERR3331365 | 3 | 3 | 4 | 2 | 3 | 3 | 3 | 209 | <i>C. diphtheriae</i> |
| ERR3932476 | 2 | 1 | 33 | 28 | 11 | 2 | 2 | 82 | <i>C. diphtheriae</i> |
| ERR3932480 | 2 | 1 | 28 | 4 | 39 | 4 | 9 | 222 | <i>C. diphtheriae</i> |
| ERR3932481 | 2 | 1 | 32 | 19 | 13 | 3 | 6 | 80 | <i>C. diphtheriae</i> |
| ERR3932482 | 2 | 10 | 17 | 13 | 40 | 2 | 4 | 223 | <i>C. diphtheriae</i> |
| ERR3932483 | 35 | 10 | 33 | 19 | 13 | 3 | 6 | 192 | <i>C. diphtheriae</i> |
| ERR3932485 | 2 | 4 | 3 | 1 | 7 | 37 | 4 | 225 | <i>C. diphtheriae</i> |
| ERR3932487 | 13 | 29 | 66 | 44 | 1 | 1 | 6 | 227 | <i>C. diphtheriae</i> |
| ERR3932488 | 2 | 1 | 2 | 1 | 7 | 3 | 9 | 233 | <i>C. diphtheriae</i> |
| ERR3932491 | 22 | 18 | 6 | 1 | 3 | 1 | 4 | 116 | <i>C. diphtheriae</i> |
| ERR3932492 | 5 | 6 | 7 | 6 | 6 | 3 | 8 | 25 | <i>C. diphtheriae</i> |
| ERR3932495 | 3 | 1 | 16 | 4 | 13 | 3 | 5 | 146 | <i>C. diphtheriae</i> |
| ERR3932496 | 2 | 9 | 4 | 1 | 30 | 28 | 2 | 131 | <i>C. diphtheriae</i> |
| ERR3932497 | 2 | 3 | 3 | 19 | 13 | 3 | 6 | 132 | <i>C. diphtheriae</i> |
| ERR3932498 | 3 | 2 | 5 | 1 | 27 | 5 | 9 | 150 | <i>C. diphtheriae</i> |
| ERR3932499 | 33 | 15 | 4 | 1 | 3 | 3 | 2 | 140 | <i>C. diphtheriae</i> |
| ERR3932500 | 1 | 2 | 9 | 7 | 8 | 3 | 2 | 183 | <i>C. diphtheriae</i> |
| ERR3932502 | 2 | 1 | 45 | 1 | 5 | 3 | 9 | 123 | <i>C. diphtheriae</i> |
| ERR3932504 | 3 | 2 | 4 | 3 | 4 | 4 | 4 | 4 | <i>C. diphtheriae</i> |
| ERR3932505 | 29 | 19 | 25 | 1 | 28 | 6 | 2 | 118 | <i>C. diphtheriae</i> |
| ERR3932506 | 2 | 1 | 33 | 28 | 11 | 2 | 3 | 147 | <i>C. diphtheriae</i> |
| ERR3932507 | 19 | 9 | 4 | 2 | 3 | 3 | 2 | 121 | <i>C. diphtheriae</i> |
| ERR3932508 | 3 | 4 | 3 | 1 | 4 | 3 | 2 | 11 | <i>C. diphtheriae</i> |
| ERR3932510 | 2 | 10 | 9 | 1 | 3 | 27 | 2 | 130 | <i>C. diphtheriae</i> |
| ERR3932511 | 19 | 8 | 11 | 9 | 3 | 3 | 5 | 665 | <i>C. diphtheriae</i> |
| ERR3932512 | 2 | 4 | 4 | 1 | 3 | 3 | 7 | 9 | <i>C. diphtheriae</i> |
| ERR3932515 | 16 | 4 | 8 | 1 | 7 | 16 | 9 | 46 | <i>C. diphtheriae</i> |
| ERR3932516 | 7 | 8 | 12 | 10 | 10 | 6 | 4 | 666 | <i>C. diphtheriae</i> |
| ERR3932517 | 32 | 22 | 45 | 5 | 18 | 3 | 6 | 133 | <i>C. diphtheriae</i> |
| ERR3932518 | 30 | 4 | 28 | 25 | 3 | 3 | 6 | 135 | <i>C. diphtheriae</i> |
| ERR3932519 | 3 | 2 | 1 | 1 | 13 | 3 | 2 | 152 | <i>C. diphtheriae</i> |
| ERR3932520 | 4 | 11 | 4 | 19 | 3 | 29 | 9 | 134 | <i>C. diphtheriae</i> |
| ERR3932521 | 36 | 10 | 3 | 1 | 7 | 3 | 22 | 165 | <i>C. diphtheriae</i> |
| ERR3932523 | 8 | 2 | 16 | 1 | 3 | 3 | 12 | 26 | <i>C. diphtheriae</i> |
| ERR3932524 | 3 | 4 | 3 | 6 | 3 | 3 | 3 | 141 | <i>C. diphtheriae</i> |
| ERR3932525 | 8 | 3 | 19 | 2 | 7 | 3 | 3 | 40 | <i>C. diphtheriae</i> |
| ERR3932526 | 4 | 1 | 49 | 5 | 11 | 3 | 6 | 148 | <i>C. diphtheriae</i> |
| ERR3932527 | 4 | 2 | 2 | 1 | 2 | 2 | 2 | 155 | <i>C. diphtheriae</i> |
| ERR3932528 | 2 | 10 | 24 | 1 | 3 | 31 | 2 | 157 | <i>C. diphtheriae</i> |
| ERR3932529 | 4 | 20 | 44 | 33 | 29 | 1 | 21 | 119 | <i>C. diphtheriae</i> |

| ID | atpA | dnaE | dnaK | fusA | leuA | odhA | rpoB | ST | Species (animal)† |
|------------|------|------|------|------|------|------|------|-----|-----------------------|
| ERR3932530 | 3 | 2 | 13 | 35 | 3 | 2 | 4 | 149 | <i>C. diphtheriae</i> |
| ERR3932532 | 14 | 2 | 23 | 4 | 2 | 14 | 2 | 44 | <i>C. diphtheriae</i> |
| ERR3932533 | 3 | 2 | 3 | 1 | 33 | 16 | 18 | 154 | <i>C. diphtheriae</i> |
| ERR3932534 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | <i>C. diphtheriae</i> |
| ERR3932537 | 3 | 5 | 6 | 5 | 3 | 3 | 6 | 8 | <i>C. diphtheriae</i> |
| ERR3932538 | 3 | 4 | 4 | 1 | 3 | 3 | 5 | 117 | <i>C. diphtheriae</i> |
| ERR3932539 | 16 | 4 | 8 | 1 | 3 | 30 | 9 | 158 | <i>C. diphtheriae</i> |
| ERR3932540 | 4 | 2 | 5 | 2 | 3 | 3 | 5 | 6 | <i>C. diphtheriae</i> |
| ERR3932541 | 32 | 1 | 45 | 5 | 11 | 3 | 6 | 145 | <i>C. diphtheriae</i> |
| ERR3932542 | 1 | 1 | 1 | 1 | 1 | 78 | 1 | 671 | <i>C. diphtheriae</i> |
| ERR3932543 | 9 | 4 | 13 | 11 | 3 | 3 | 9 | 21 | <i>C. diphtheriae</i> |
| ERR3932545 | 4 | 21 | 2 | 1 | 3 | 3 | 3 | 127 | <i>C. diphtheriae</i> |
| ERR3932546 | 31 | 4 | 48 | 1 | 7 | 1 | 9 | 129 | <i>C. diphtheriae</i> |
| ERR3932547 | 2 | 4 | 8 | 1 | 7 | 3 | 9 | 258 | <i>C. diphtheriae</i> |
| ERR3932548 | 19 | 9 | 46 | 19 | 3 | 26 | 22 | 126 | <i>C. diphtheriae</i> |
| ERR3932549 | 22 | 4 | 138 | 1 | 18 | 3 | 4 | 672 | <i>C. diphtheriae</i> |
| ERR3932554 | 30 | 4 | 35 | 25 | 11 | 3 | 6 | 124 | <i>C. diphtheriae</i> |
| ERR3932555 | 2 | 10 | 29 | 1 | 12 | 2 | 13 | 143 | <i>C. diphtheriae</i> |
| ERR3932557 | 2 | 1 | 39 | 19 | 13 | 24 | 13 | 96 | <i>C. diphtheriae</i> |
| ERR3932558 | 2 | 1 | 6 | 29 | 3 | 3 | 2 | 91 | <i>C. diphtheriae</i> |
| ERR3932560 | 3 | 1 | 5 | 2 | 3 | 23 | 2 | 88 | <i>C. diphtheriae</i> |
| ERR3932564 | 2 | 1 | 38 | 29 | 3 | 3 | 2 | 87 | <i>C. diphtheriae</i> |
| ERR3932568 | 19 | 10 | 61 | 4 | 5 | 5 | 2 | 206 | <i>C. diphtheriae</i> |
| ERR3932569 | 2 | 4 | 4 | 29 | 3 | 16 | 2 | 205 | <i>C. diphtheriae</i> |
| ERR3932570 | 3 | 1 | 13 | 16 | 38 | 2 | 9 | 212 | <i>C. diphtheriae</i> |
| ERR3932574 | 2 | 10 | 56 | 45 | 11 | 3 | 3 | 228 | <i>C. diphtheriae</i> |
| ERR3932575 | 2 | 1 | 9 | 19 | 3 | 17 | 2 | 667 | <i>C. diphtheriae</i> |
| ERR3932576 | 2 | 1 | 9 | 29 | 3 | 3 | 2 | 237 | <i>C. diphtheriae</i> |
| ERR3932579 | 3 | 4 | 67 | 29 | 3 | 16 | 2 | 235 | <i>C. diphtheriae</i> |
| ERR3932580 | 2 | 9 | 6 | 29 | 3 | 3 | 2 | 238 | <i>C. diphtheriae</i> |
| ERR3932581 | 11 | 1 | 20 | 16 | 3 | 3 | 14 | 239 | <i>C. diphtheriae</i> |
| ERR3932584 | 2 | 9 | 9 | 29 | 3 | 3 | 2 | 241 | <i>C. diphtheriae</i> |
| ERR3932585 | 32 | 1 | 70 | 19 | 3 | 3 | 18 | 250 | <i>C. diphtheriae</i> |
| ERR3932587 | 13 | 2 | 44 | 41 | 29 | 1 | 21 | 366 | <i>C. diphtheriae</i> |
| ERR3932589 | 2 | 9 | 4 | 59 | 3 | 3 | 2 | 424 | <i>C. diphtheriae</i> |
| ERR3932590 | 3 | 2 | 4 | 1 | 3 | 3 | 13 | 136 | <i>C. diphtheriae</i> |
| ERR3932594 | 3 | 4 | 11 | 2 | 3 | 3 | 2 | 426 | <i>C. diphtheriae</i> |
| ERR3932595 | 22 | 2 | 4 | 29 | 3 | 40 | 2 | 268 | <i>C. diphtheriae</i> |
| ERR3932600 | 3 | 2 | 73 | 4 | 43 | 22 | 2 | 274 | <i>C. diphtheriae</i> |
| ERR3932601 | 28 | 2 | 38 | 19 | 13 | 3 | 6 | 269 | <i>C. diphtheriae</i> |
| ERR3932602 | 2 | 8 | 63 | 30 | 85 | 3 | 19 | 673 | <i>C. diphtheriae</i> |
| ERR3932603 | 2 | 10 | 69 | 4 | 5 | 11 | 2 | 668 | <i>C. diphtheriae</i> |
| ERR3932604 | 4 | 4 | 96 | 7 | 86 | 57 | 32 | 674 | <i>C. diphtheriae</i> |
| ERR3932606 | 11 | 1 | 85 | 2 | 3 | 3 | 2 | 356 | <i>C. diphtheriae</i> |
| ERR3932608 | 22 | 9 | 43 | 7 | 3 | 3 | 2 | 109 | <i>C. diphtheriae</i> |
| ERR3932611 | 4 | 1 | 20 | 19 | 13 | 16 | 9 | 361 | <i>C. diphtheriae</i> |
| ERR3932612 | 25 | 1 | 6 | 19 | 3 | 16 | 6 | 425 | <i>C. diphtheriae</i> |
| ERR3932613 | 2 | 23 | 32 | 1 | 55 | 5 | 43 | 362 | <i>C. diphtheriae</i> |
| ERR3932614 | 28 | 12 | 6 | 19 | 3 | 51 | 6 | 352 | <i>C. diphtheriae</i> |
| ERR3932616 | 2 | 1 | 61 | 19 | 3 | 16 | 2 | 355 | <i>C. diphtheriae</i> |
| ERR3932617 | 24 | 15 | 86 | 19 | 11 | 3 | 18 | 374 | <i>C. diphtheriae</i> |
| ERR3932618 | 3 | 2 | 35 | 1 | 18 | 3 | 2 | 86 | <i>C. diphtheriae</i> |
| ERR3932621 | 46 | 10 | 60 | 7 | 3 | 3 | 2 | 375 | <i>C. diphtheriae</i> |
| ERR3932624 | 3 | 8 | 9 | 1 | 3 | 40 | 2 | 310 | <i>C. diphtheriae</i> |
| ERR3932625 | 3 | 1 | 12 | 1 | 42 | 16 | 31 | 259 | <i>C. diphtheriae</i> |
| ERR3932626 | 2 | 26 | 4 | 4 | 62 | 3 | 2 | 421 | <i>C. diphtheriae</i> |
| ERR3932627 | 3 | 8 | 8 | 30 | 3 | 4 | 19 | 95 | <i>C. diphtheriae</i> |
| ERR3932630 | 1 | 2 | 9 | 57 | 3 | 3 | 2 | 419 | <i>C. diphtheriae</i> |
| ERR3932632 | 3 | 2 | 70 | 1 | 7 | 3 | 2 | 595 | <i>C. diphtheriae</i> |
| ERR3932633 | 3 | 3 | 92 | 2 | 3 | 3 | 3 | 387 | <i>C. diphtheriae</i> |
| ERR3932634 | 2 | 2 | 38 | 58 | 3 | 3 | 22 | 422 | <i>C. diphtheriae</i> |
| ERR3932636 | 18 | 10 | 27 | 21 | 20 | 5 | 16 | 669 | <i>C. diphtheriae</i> |
| ERR3932638 | 2 | 2 | 4 | 1 | 3 | 3 | 46 | 388 | <i>C. diphtheriae</i> |
| ERR3932639 | 3 | 8 | 9 | 30 | 3 | 4 | 19 | 413 | <i>C. diphtheriae</i> |
| ERR3932640 | 2 | 1 | 61 | 4 | 5 | 5 | 2 | 423 | <i>C. diphtheriae</i> |
| ERR3932642 | 3 | 1 | 38 | 4 | 3 | 3 | 2 | 410 | <i>C. diphtheriae</i> |
| ERR3932643 | 2 | 2 | 2 | 3 | 11 | 64 | 4 | 536 | <i>C. diphtheriae</i> |
| ERR3932644 | 4 | 2 | 104 | 30 | 3 | 51 | 2 | 511 | <i>C. diphtheriae</i> |
| ERR3932645 | 2 | 26 | 55 | 29 | 30 | 3 | 2 | 414 | <i>C. diphtheriae</i> |

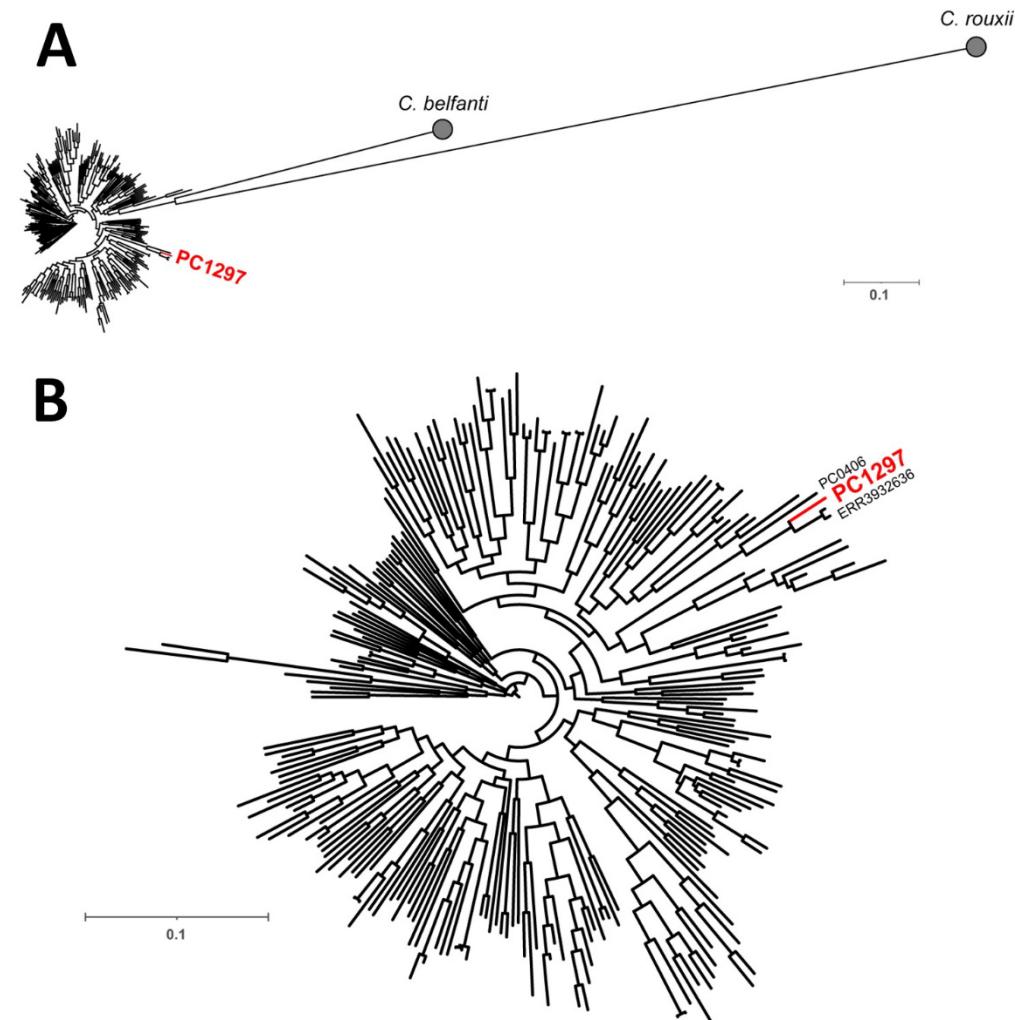
| ID | atpA | dnaE | dnaK | fusA | leuA | odhA | rpoB | ST | Species (animal)† |
|-------------|------|------|------|------|------|------|------|-----|-----------------------|
| ERR3932647 | 22 | 2 | 4 | 16 | 3 | 40 | 2 | 415 | <i>C. diphtheriae</i> |
| ERR3932648 | 25 | 16 | 35 | 44 | 3 | 3 | 28 | 416 | <i>C. diphtheriae</i> |
| ERR3932649 | 2 | 2 | 42 | 31 | 11 | 3 | 4 | 100 | <i>C. diphtheriae</i> |
| ERR3932651 | 2 | 8 | 32 | 19 | 11 | 17 | 18 | 429 | <i>C. diphtheriae</i> |
| ERR3932652 | 19 | 1 | 95 | 2 | 3 | 16 | 6 | 420 | <i>C. diphtheriae</i> |
| ERR3932653 | 2 | 8 | 4 | 1 | 3 | 3 | 6 | 430 | <i>C. diphtheriae</i> |
| ERR3932655 | 4 | 1 | 2 | 19 | 25 | 16 | 18 | 101 | <i>C. diphtheriae</i> |
| ERR3932656 | 4 | 12 | 4 | 1 | 18 | 3 | 13 | 308 | <i>C. diphtheriae</i> |
| ERR3932657 | 3 | 9 | 4 | 1 | 3 | 3 | 5 | 417 | <i>C. diphtheriae</i> |
| ERR3932658 | 2 | 1 | 60 | 4 | 3 | 3 | 18 | 431 | <i>C. diphtheriae</i> |
| ERR3932660 | 30 | 2 | 32 | 19 | 24 | 16 | 9 | 418 | <i>C. diphtheriae</i> |
| ERR3932661 | 2 | 1 | 20 | 19 | 3 | 3 | 14 | 295 | <i>C. diphtheriae</i> |
| ERR3932662 | 2 | 2 | 92 | 13 | 8 | 3 | 5 | 411 | <i>C. diphtheriae</i> |
| ERR3932667 | 19 | 32 | 38 | 82 | 44 | 2 | 20 | 675 | <i>C. diphtheriae</i> |
| ERR3932668 | 1 | 2 | 9 | 7 | 73 | 3 | 2 | 549 | <i>C. diphtheriae</i> |
| ERR3932673 | 1 | 48 | 139 | 7 | 3 | 57 | 9 | 676 | <i>C. diphtheriae</i> |
| ERR3932674 | 13 | 16 | 35 | 44 | 29 | 3 | 28 | 517 | <i>C. diphtheriae</i> |
| ERR3932676 | 2 | 1 | 6 | 1 | 3 | 40 | 14 | 481 | <i>C. diphtheriae</i> |
| ERR3932678 | 2 | 2 | 3 | 2 | 3 | 3 | 5 | 519 | <i>C. diphtheriae</i> |
| ERR3932680 | 3 | 5 | 5 | 2 | 3 | 23 | 20 | 520 | <i>C. diphtheriae</i> |
| ERR3932681 | 2 | 1 | 111 | 1 | 5 | 40 | 2 | 532 | <i>C. diphtheriae</i> |
| ERR3932682 | 48 | 44 | 88 | 1 | 60 | 1 | 6 | 380 | <i>C. diphtheriae</i> |
| ERR3932685 | 33 | 57 | 112 | 44 | 3 | 1 | 13 | 533 | <i>C. diphtheriae</i> |
| ERR3932686 | 1 | 1 | 32 | 1 | 70 | 5 | 30 | 522 | <i>C. diphtheriae</i> |
| ERR3932687 | 4 | 55 | 5 | 19 | 5 | 59 | 2 | 521 | <i>C. diphtheriae</i> |
| ERR3932690 | 2 | 1 | 55 | 25 | 55 | 3 | 2 | 490 | <i>C. diphtheriae</i> |
| ERR3932693 | 13 | 29 | 108 | 64 | 60 | 1 | 23 | 524 | <i>C. diphtheriae</i> |
| ERR3932697 | 2 | 1 | 47 | 19 | 13 | 3 | 6 | 128 | <i>C. diphtheriae</i> |
| ERR3932700 | 2 | 4 | 101 | 7 | 3 | 3 | 32 | 487 | <i>C. diphtheriae</i> |
| ERR3932701 | 28 | 9 | 4 | 64 | 3 | 3 | 2 | 526 | <i>C. diphtheriae</i> |
| ERR3932702 | 4 | 2 | 109 | 65 | 3 | 51 | 2 | 527 | <i>C. diphtheriae</i> |
| ERR3932705 | 5 | 2 | 70 | 19 | 33 | 3 | 53 | 530 | <i>C. diphtheriae</i> |
| ERR3932707 | 3 | 1 | 9 | 5 | 3 | 4 | 36 | 320 | <i>C. diphtheriae</i> |
| ERR3932708 | 3 | 2 | 73 | 4 | 4 | 22 | 4 | 427 | <i>C. diphtheriae</i> |
| ERR4332857 | 3 | 1 | 20 | 7 | 13 | 54 | 2 | 724 | <i>C. diphtheriae</i> |
| ERR4332868 | 3 | 1 | 20 | 2 | 13 | 54 | 2 | 384 | <i>C. diphtheriae</i> |
| ERR4332886 | 4 | 46 | 4 | 1 | 3 | 3 | 2 | 447 | <i>C. diphtheriae</i> |
| ERR4332890 | 2 | 9 | 96 | 2 | 3 | 3 | 4 | 725 | <i>C. diphtheriae</i> |
| ERR4332892 | 2 | 12 | 4 | 1 | 3 | 3 | 2 | 292 | <i>C. diphtheriae</i> |
| SRR10054224 | 2 | 9 | 1 | 1 | 13 | 3 | 2 | 76 | <i>C. diphtheriae</i> |
| SRR10054230 | 3 | 1 | 150 | 19 | 13 | 3 | 48 | 739 | <i>C. diphtheriae</i> |
| SRR11184121 | 3 | 1 | 107 | 19 | 13 | 3 | 48 | 652 | <i>C. diphtheriae</i> |
| SRR11184124 | 2 | 4 | 6 | 19 | 13 | 3 | 6 | 625 | <i>C. diphtheriae</i> |
| SRR11184125 | 2 | 1 | 76 | 19 | 13 | 24 | 13 | 317 | <i>C. diphtheriae</i> |
| SRR11184130 | 2 | 1 | 20 | 19 | 42 | 16 | 13 | 244 | <i>C. diphtheriae</i> |
| SRR11184131 | 2 | 4 | 137 | 81 | 13 | 3 | 6 | 662 | <i>C. diphtheriae</i> |
| SRR11184133 | 3 | 46 | 3 | 6 | 30 | 3 | 2 | 439 | <i>C. diphtheriae</i> |
| SRR11184136 | 38 | 1 | 77 | 4 | 5 | 3 | 9 | 319 | <i>C. diphtheriae</i> |
| SRR11184138 | 33 | 2 | 4 | 1 | 3 | 3 | 2 | 655 | <i>C. diphtheriae</i> |
| SRR11184142 | 3 | 1 | 45 | 1 | 5 | 3 | 9 | 654 | <i>C. diphtheriae</i> |
| SRR11184144 | 4 | 1 | 136 | 5 | 11 | 1 | 6 | 661 | <i>C. diphtheriae</i> |
| SRR11184153 | 3 | 2 | 131 | 71 | 7 | 2 | 4 | 653 | <i>C. diphtheriae</i> |
| SRR11184159 | 2 | 1 | 35 | 29 | 3 | 3 | 2 | 623 | <i>C. diphtheriae</i> |
| SRR11184162 | 2 | 1 | 45 | 46 | 5 | 16 | 9 | 246 | <i>C. diphtheriae</i> |
| SRR11184166 | 2 | 3 | 92 | 2 | 3 | 3 | 3 | 658 | <i>C. diphtheriae</i> |
| SRR11184167 | 3 | 1 | 18 | 4 | 13 | 3 | 5 | 32 | <i>C. diphtheriae</i> |
| SRR11184172 | 2 | 3 | 38 | 64 | 7 | 3 | 2 | 657 | <i>C. diphtheriae</i> |
| SRR11184173 | 2 | 1 | 35 | 19 | 13 | 24 | 13 | 257 | <i>C. diphtheriae</i> |
| SRR11184176 | 1 | 9 | 46 | 19 | 3 | 3 | 22 | 656 | <i>C. diphtheriae</i> |
| SRR12235651 | 2 | 2 | 4 | 1 | 3 | 3 | 2 | 50 | <i>C. diphtheriae</i> |
| (PC0112) | | | | | | | | | |
| SRR12235663 | 3 | 2 | 4 | 15 | 4 | 4 | 4 | 31 | <i>C. diphtheriae</i> |
| SRR12235668 | 2 | 10 | 24 | 1 | 3 | 3 | 2 | 53 | <i>C. diphtheriae</i> |
| (PC0153) | | | | | | | | | |
| SRR12235672 | 2 | 10 | 29 | 1 | 12 | 10 | 13 | 38 | <i>C. diphtheriae</i> |
| SRR12235677 | 10 | 8 | 16 | 14 | 10 | 3 | 9 | 30 | <i>C. diphtheriae</i> |
| (PC0104) | | | | | | | | | |

| ID | atpA | dnaE | dnaK | fusA | leuA | odhA | rpoB | ST | Species (animal)† |
|-------------------------|------|------|------|------|------|------|------|-----|-----------------------------|
| SRR12270034 (PC0652) | 3 | 2 | 60 | 83 | 3 | 3 | 2 | NT | <i>C. diphtheriae</i> |
| SRR12270040 (PC0646) | 3 | 2 | 60 | 30 | 3 | 3 | 2 | 445 | <i>C. diphtheriae</i> |
| SRR6816560 | 59 | 4 | 8 | 53 | 3 | 5 | 13 | 0 | <i>C. diphtheriae</i> |
| SRR6816561 | 1 | 16 | 32 | 1 | 87 | 1 | 4 | 687 | <i>C. diphtheriae</i> |
| SRR6816562 | 49 | 4 | 8 | 53 | 3 | 5 | 13 | 729 | <i>C. diphtheriae</i> |
| SRR6816563 | 32 | 1 | 45 | 45 | 11 | 3 | 3 | 240 | <i>C. diphtheriae</i> |
| SRR6816564 | 3 | 4 | 8 | 1 | 18 | 3 | 9 | 498 | <i>C. diphtheriae</i> |
| SRR6816565 | 2 | 4 | 4 | 1 | 3 | 3 | 5 | 5 | <i>C. diphtheriae</i> |
| SRR6816566 | 1 | 1 | 88 | 44 | 97 | 89 | 13 | 749 | <i>C. diphtheriae</i> |
| SRR6816567 | 13 | 29 | 108 | 64 | 60 | 1 | 65 | 744 | <i>C. diphtheriae</i> |
| SRR6816568 | 2 | 1 | 33 | 28 | 11 | 2 | 22 | 516 | <i>C. diphtheriae</i> |
| SRR6816570 | 4 | 1 | 8 | 1 | 11 | 3 | 6 | 122 | <i>C. diphtheriae</i> |
| SRR6816572 | 18 | 1 | 27 | 21 | 20 | 5 | 16 | 59 | <i>C. diphtheriae</i> |
| SRR6816573 | 1 | 44 | 9 | 1 | 26 | 69 | 4 | 605 | <i>C. diphtheriae</i> |
| SRR6816574 | 13 | 2 | 46 | 19 | 60 | 26 | 13 | 730 | <i>C. diphtheriae</i> |
| SRR6816576 | 19 | 1 | 9 | 1 | 18 | 88 | 13 | 745 | <i>C. diphtheriae</i> |
| SRR6816578 | 2 | 1 | 35 | 4 | 37 | 3 | 9 | 731 | <i>C. diphtheriae</i> |
| SRR6816579 | 25 | 1 | 33 | 28 | 11 | 3 | 22 | 737 | <i>C. diphtheriae</i> |
| SRR6816582 | 9 | 44 | 88 | 1 | 60 | 1 | 6 | 734 | <i>C. diphtheriae</i> |
| SRR6816583 | 1 | 2 | 66 | 88 | 93 | 5 | 21 | 738 | <i>C. diphtheriae</i> |
| SRR6816585 | 2 | 2 | 13 | 1 | 3 | 3 | 13 | 727 | <i>C. diphtheriae</i> |
| SRR6816587 | 13 | 2 | 44 | 1 | 95 | 1 | 13 | 747 | <i>C. diphtheriae</i> |
| SRR6816590 | 13 | 2 | 32 | 33 | 93 | 1 | 21 | 748 | <i>C. diphtheriae</i> |
| SRR6816591 | 49 | 4 | 9 | 53 | 3 | 5 | 13 | 381 | <i>C. diphtheriae</i> |
| SRR6816592 | 25 | 16 | 35 | 46 | 2 | 3 | 4 | 732 | <i>C. diphtheriae</i> |
| SRR6816596 | 13 | 1 | 20 | 1 | 3 | 5 | 2 | 733 | <i>C. diphtheriae</i> |
| SRR6816598 | 3 | 1 | 3 | 1 | 3 | 5 | 13 | 735 | <i>C. diphtheriae</i> |
| SRR6816599 | 3 | 1 | 152 | 4 | 13 | 3 | 5 | 750 | <i>C. diphtheriae</i> |
| SRR6816601 | 8 | 3 | 3 | 2 | 7 | 3 | 3 | 20 | <i>C. diphtheriae</i> |
| SRR6816606 | 1 | 1 | 33 | 1 | 13 | 3 | 6 | 736 | <i>C. diphtheriae</i> |
| SRR7039157 | 2 | 12 | 54 | 13 | 3 | 3 | 4 | 297 | <i>C. diphtheriae</i> |
| SRR7039160 | 3 | 8 | 94 | 4 | 3 | 4 | 2 | 494 | <i>C. diphtheriae</i> |
| SRR7039165 | 19 | 11 | 4 | 19 | 3 | 29 | 9 | 495 | <i>C. diphtheriae</i> |
| SRR7039167 | 4 | 2 | 4 | 1 | 3 | 3 | 2 | 446 | <i>C. diphtheriae</i> |
| SRR7039176 | 4 | 2 | 4 | 19 | 3 | 16 | 2 | 496 | <i>C. diphtheriae</i> |
| SRR7039185 | 3 | 2 | 4 | 1 | 3 | 3 | 2 | 389 | <i>C. diphtheriae</i> |
| SRR7039187 | 3 | 1 | 4 | 19 | 13 | 3 | 48 | 441 | <i>C. diphtheriae</i> |
| SRR7039194 | 2 | 1 | 20 | 19 | 69 | 3 | 14 | 509 | <i>C. diphtheriae</i> |
| SRR7039204 | 2 | 1 | 32 | 19 | 13 | 3 | 50 | 508 | <i>C. diphtheriae</i> |
| SRR7039222 | 3 | 1 | 12 | 1 | 68 | 3 | 31 | 507 | <i>C. diphtheriae</i> |
| SRR7223801 | 19 | 4 | 8 | 1 | 3 | 3 | 13 | 125 | <i>C. diphtheriae</i> |
| SRR72825350 | 22 | 54 | 104 | 16 | 3 | 2 | 2 | 512 | <i>C. diphtheriae</i> |
| SRR72825351 | 4 | 4 | 105 | 1 | 3 | 3 | 2 | 513 | <i>C. diphtheriae</i> |
| SRR72825371 | 8 | 8 | 3 | 1 | 7 | 3 | 2 | 497 | <i>C. diphtheriae</i> |
| SRR72825427 | 3 | 1 | 94 | 1 | 3 | 3 | 2 | 500 | <i>C. diphtheriae</i> |
| SRR8417825 | 2 | 12 | 125 | 1 | 3 | 3 | 2 | 586 | <i>C. diphtheriae</i> |
| PC1297 | 18 | 10 | 30 | 21 | 20 | 5 | 16 | 705 | <i>C. diphtheriae (cat)</i> |
| PC0406 | 3 | 1 | 110 | 19 | 20 | 61 | 16 | 528 | <i>C. diphtheriae (dog)</i> |
| ERR3574992 | 37 | 25 | 53 | 39 | 36 | 35 | 17 | 181 | <i>C. rouxii</i> |
| ERR3574995 | 37 | 25 | 113 | 66 | 72 | 35 | 17 | 538 | <i>C. rouxii</i> |
| SRR12235660 (PC0231) | 20 | 14 | 31 | 26 | 22 | 21 | 17 | 74 | <i>C. rouxii (cat)</i> |
| SRR12235661 (PC0230) | 20 | 14 | 31 | 26 | 22 | 21 | 17 | 74 | <i>C. rouxii (cat)</i> |
| SRR12235662 (PC0229) | 20 | 14 | 31 | 26 | 22 | 21 | 17 | 74 | <i>C. rouxii (cat)</i> |
| SRR12235664 (PC0226) | 20 | 14 | 31 | 26 | 22 | 21 | 17 | 74 | <i>C. rouxii (cat)</i> |
| PC0751 | 37 | 25 | 91 | 54 | 61 | 21 | 17 | 537 | <i>C. rouxii (dog)</i> |
| PC1315 | 37 | 25 | 91 | 26 | 83 | 35 | 3 | 0 | <i>C. rouxii (dog)</i> |
| ERR2094320 | 6 | 17 | 10 | 12 | 9 | 7 | 11 | 107 | <i>C. belfanti</i> |
| ERR2094321 | 6 | 7 | 10 | 8 | 34 | 13 | 10 | 177 | <i>C. belfanti</i> |
| ERR2094323 | 6 | 7 | 10 | 12 | 9 | 12 | 10 | 106 | <i>C. belfanti</i> |
| ERR2094328 | 6 | 7 | 10 | 18 | 56 | 12 | 11 | 365 | <i>C. belfanti</i> |
| ERR2094329 | 6 | 7 | 10 | 8 | 9 | 13 | 10 | 92 | <i>C. belfanti</i> |
| ERR2757918 | 6 | 7 | 62 | 37 | 9 | 12 | 10 | 208 | <i>C. belfanti</i> |
| SRR11184122 | 6 | 7 | 134 | 63 | 9 | 7 | 10 | 659 | <i>C. belfanti</i> |

| ID | atpA | dnaE | dnaK | fusA | leuA | odhA | rpoB | ST | Species (animal)† |
|-------------|------|------|------|------|------|------|------|-----|--------------------|
| SRR11184123 | 6 | 7 | 10 | 12 | 9 | 7 | 10 | 81 | <i>C. belfanti</i> |
| SRR11184129 | 6 | 7 | 10 | 12 | 84 | 13 | 15 | 663 | <i>C. belfanti</i> |
| SRR11184141 | 6 | 7 | 10 | 18 | 9 | 13 | 10 | 170 | <i>C. belfanti</i> |
| SRR11184154 | 6 | 7 | 135 | 8 | 9 | 77 | 10 | 660 | <i>C. belfanti</i> |
| SRR11184155 | 6 | 7 | 21 | 12 | 9 | 12 | 11 | 409 | <i>C. belfanti</i> |
| SRR11184157 | 6 | 7 | 10 | 37 | 9 | 12 | 11 | 353 | <i>C. belfanti</i> |
| SRR11184171 | 6 | 7 | 10 | 37 | 9 | 12 | 10 | 226 | <i>C. belfanti</i> |
| SRR7039177 | 6 | 7 | 21 | 61 | 9 | 7 | 10 | 501 | <i>C. belfanti</i> |
| SRR7039197 | 6 | 7 | 10 | 8 | 9 | 12 | 11 | 294 | <i>C. belfanti</i> |

ST, sequence type

†When applicable



Appendix Figure. Phylogenetic reconstruction of *C. diphtheriae* PC1297 and 273 publicly available isolate sequences from 252,766 core SNP sites using maximum likelihood. Cat isolate PC1297 A) was placed within the main clade of typical *C. diphtheriae* strains, distinct from related novel species *C. belfanti* and *C. rouxii* previously associated with domestic dog and cat isolates, and B) was most closely related to human isolate ERR3932636 and shared a branch with dog isolate PC0406. Scale bars indicates substitutions per site.