Genome contamination has minimal impact on the delineation of species

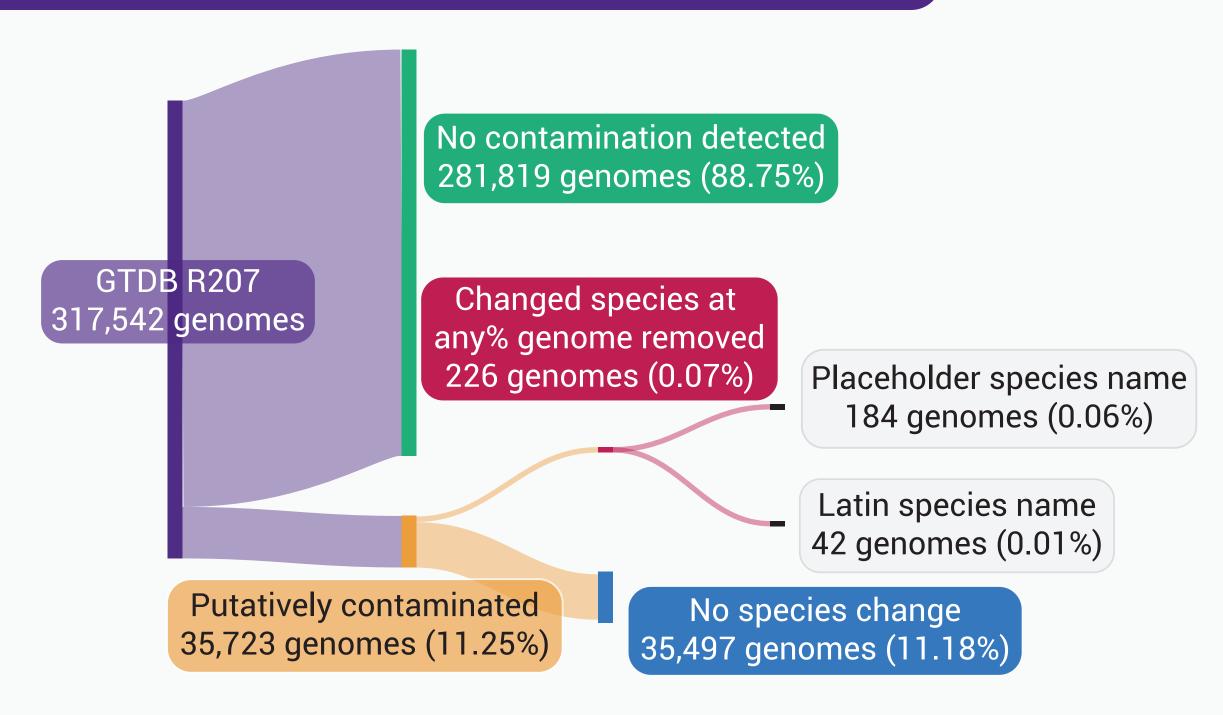
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Contamination is a known issue, but to what extent does this impact taxonomy?

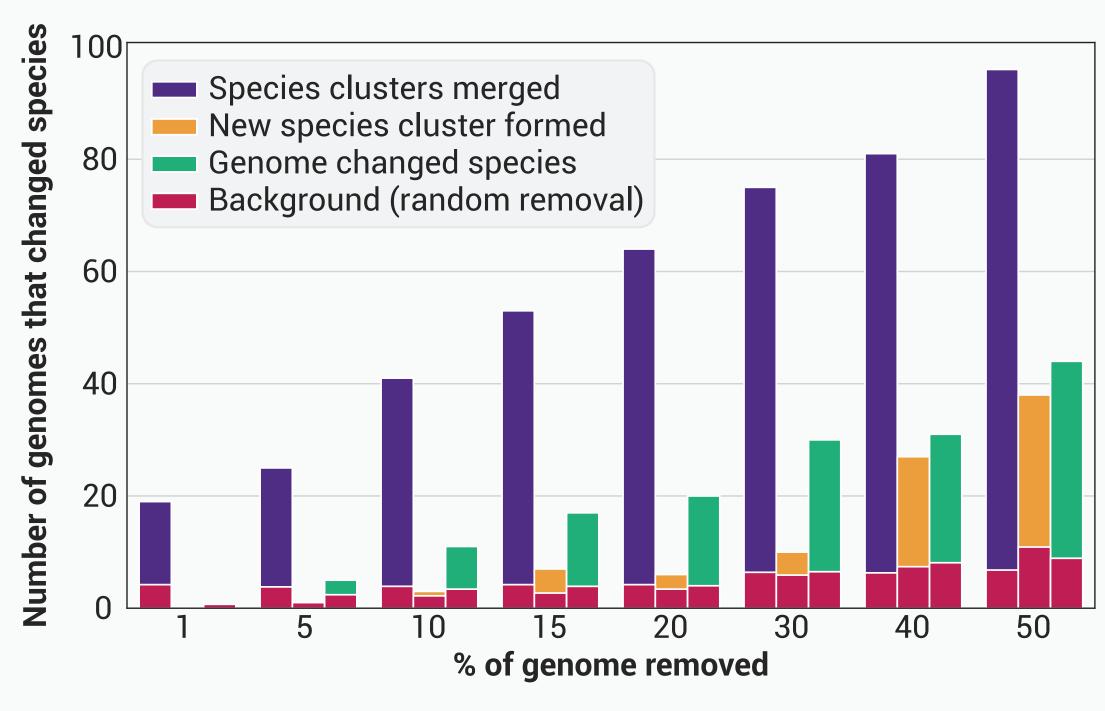
We assess the impact of putative contamination on species delineated using average nucleotide identity (ANI).

Species clustering is robust to contamination



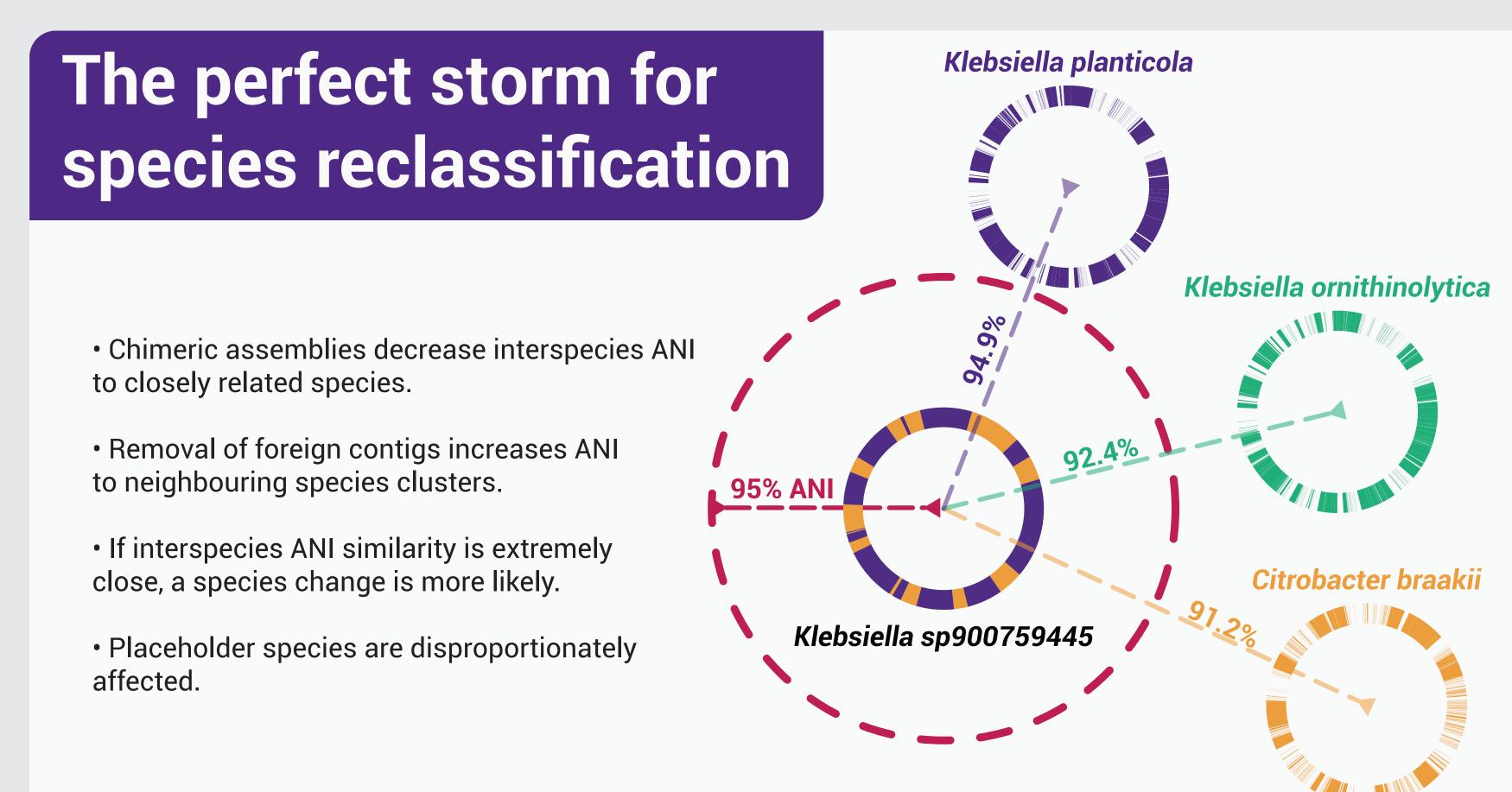
- 35,723 of 317,542 (11.25%) GTDB R207 genomes were putatively contaminated.
- Identifying contamination using a reference database has limitations:
- i) Taxonomically novel genomes are insufficiently represented. ii) Order that genomes appear in the GUNC reference database impacts results.

Up to 226 genomes (0.07%) change species assignment when removing putatively contaminated contigs

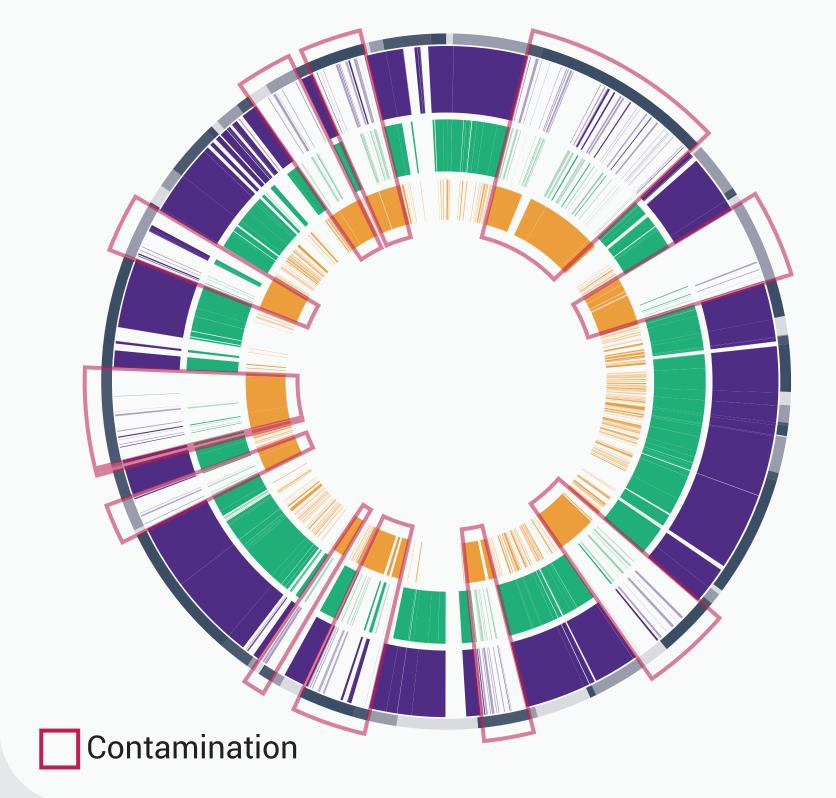


- Even with removing half of the genome, there were only 226 genome that changed species.
- 184 of the genome changes were in nomenclaturely unimportant species (i.e. placeholder species).
- The 226 genomes belonged to 206 unique species clusters from 134 unique genera.

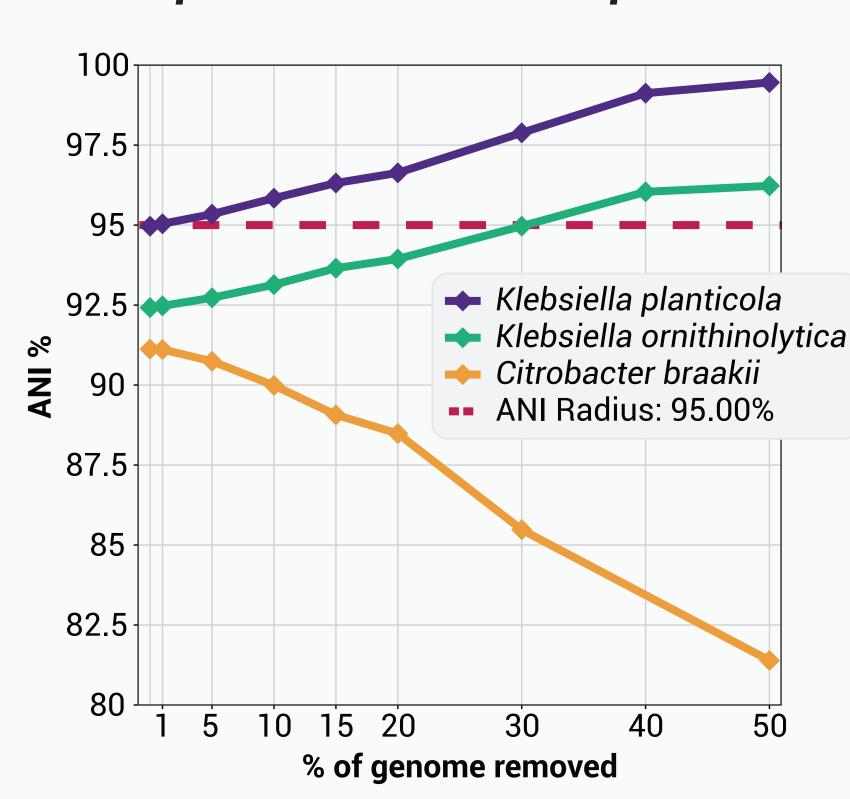
Method **GTDB R207** 317,542 genomes **Run GUNC Run GUNC Randomly select genomes** (GTDB R95 reference database) (proGenomes 2.1 reference database) n=35,723 with 10 repetitions 17,278 putatively contaminated genomes 28,858 putatively contaminated genomes Take hit with highest contamination score 35,723 putatively contaminated genomes Order contigs from most to least contaminated Randomly order contigs Remove contigs in order until x% of genome removed $x \in \{1, 5, 10, 15, 20, 30, 40, 50\}$ Establish background Infer species clusters using ANI Identify contamination



Whole genome alignment shows C. braakii contamination



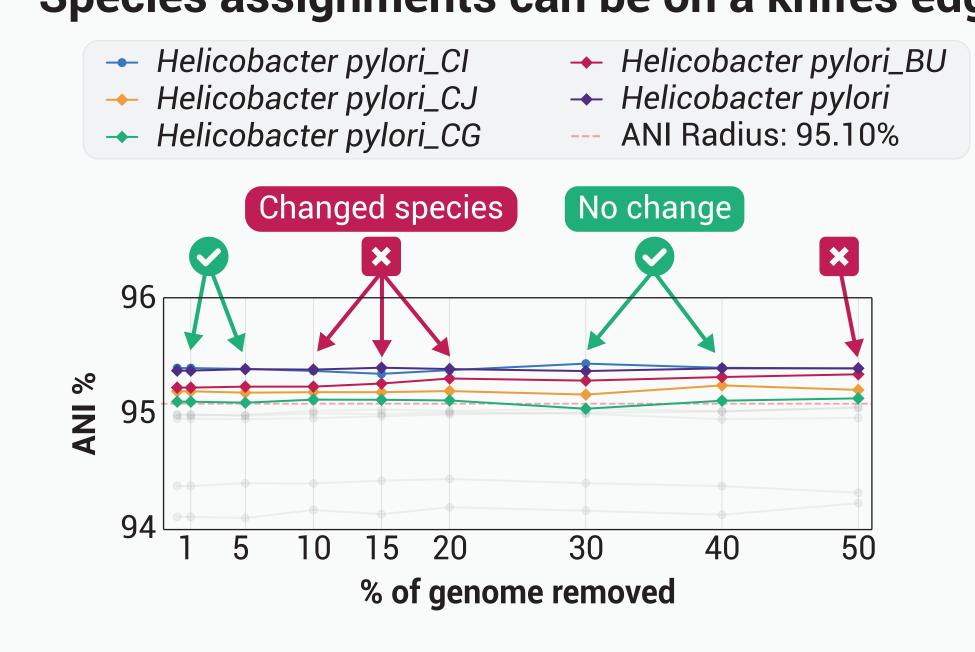
Removing contamination merges *K. sp900759445* and *K. planticola*



Closely related genomes blur interspecies boundaries

- 76.75% of the interspecies ANI comparisons were >92% ANI for the 226 genomes that changed species, compared to 16.58% for all genomes in GTDB R207.
- Significantly more non-representative genomes fall within multiple species ANI radii.
- Genomes are more likely to have a species reassignment if they are close to multiple representatives.

Species assignments can be on a knifes edge



Interspecies ANI is higher for

