GTDB-Tk 2: memory friendly classification with the Genome Taxonomy Database

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What is GTDB-Tk?

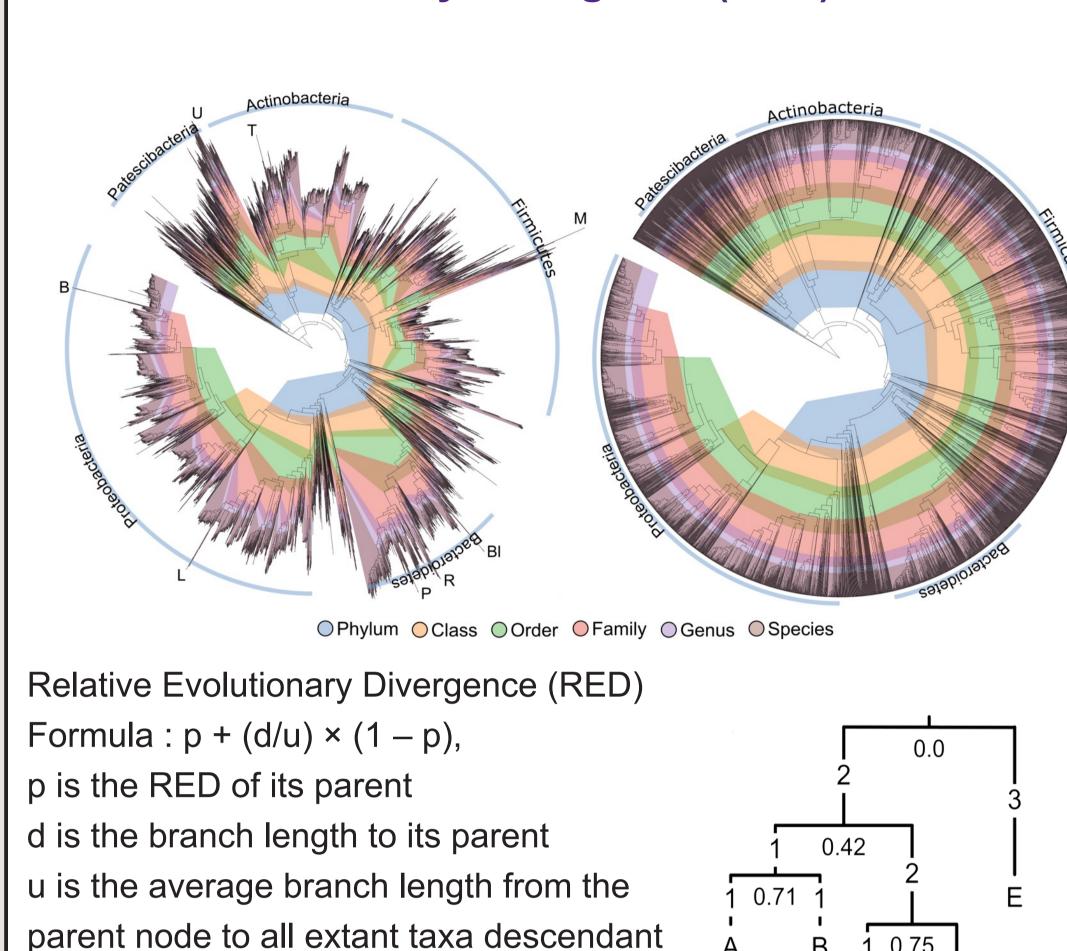
The Genome Taxonomy Database Toolkit (GTDB-Tk) is:

- Computationally efficient
- Provides automated and objective taxonomic classification of bacterial and archaeal genomes
- Places genomes into domain-specific, concatenated protein reference trees.
- Used to assign taxonomic classifications to tens of thousands of bacterial and archaeal metagenome-assemble genomes (MAGs) recovered from environmental and human-associated samples (*Chaumeil et al., 2019; Almeida et al., 2021; Nayfach et al., 2021; Chen et al., 2021*).

Why do we need GTDB-Tk v2?

- GTDB-Tk is placing genomes into the GTDB reference trees using the maximum-likelihood (ML) placement tool pplacer (Matsen et al. 2010).
- When using the GTDB R07-RS207 bacterial reference tree comprised of 62,291 genomes, pplacer requires
 ~320 GB of RAM .
- GTDB-Tk v2 addresses the memory requirements by dividing the GTDB bacterial reference tree into class-level subtrees.

Genomes **Taxon Novelty Novel phylum Novel class Novel order** Classify using backbone tree **Novel family** Novel genus **Novel species** Does genome have a class level classification? No, Yes Assign genome to class-level tree Establish taxonomy using RED Establish taxonomy using **RED** and ANI Compare backbone and class-level taxonomies Final taxonomy **Performance**



GTDB-Tk v2 classifications relative to GTDB-Tk v1 classifications

Conflict

No. genomes

144

543

3,222

12,756

from node to calculate.

Example, the parent node of leaves C and D

has a RED value of $0.75 = (0.42 + (2/3.5) \times (1 - 0.42))$

Congruent

143

540

3,219

12,576

Relative Evolutionary Divergence (RED)

Underclassified

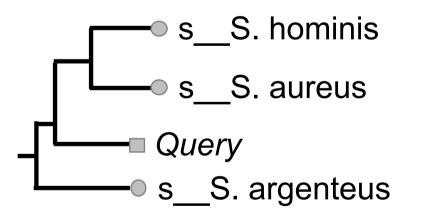
Overclassified

How does GTDB-Tk classify my genomes? Query p_Proteobacteria Query genome represents a new phylum. c_Coriobacteriia Query c_Actinobacteria c_Actinobacteria

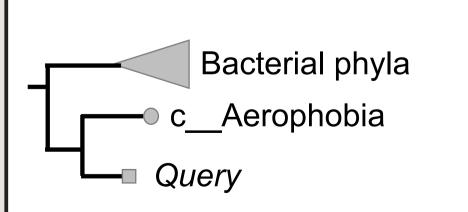
Query genome represents a novel class within the phylum *Actinobacteria*.

g_Citrobacter
g_Escherichia
Query

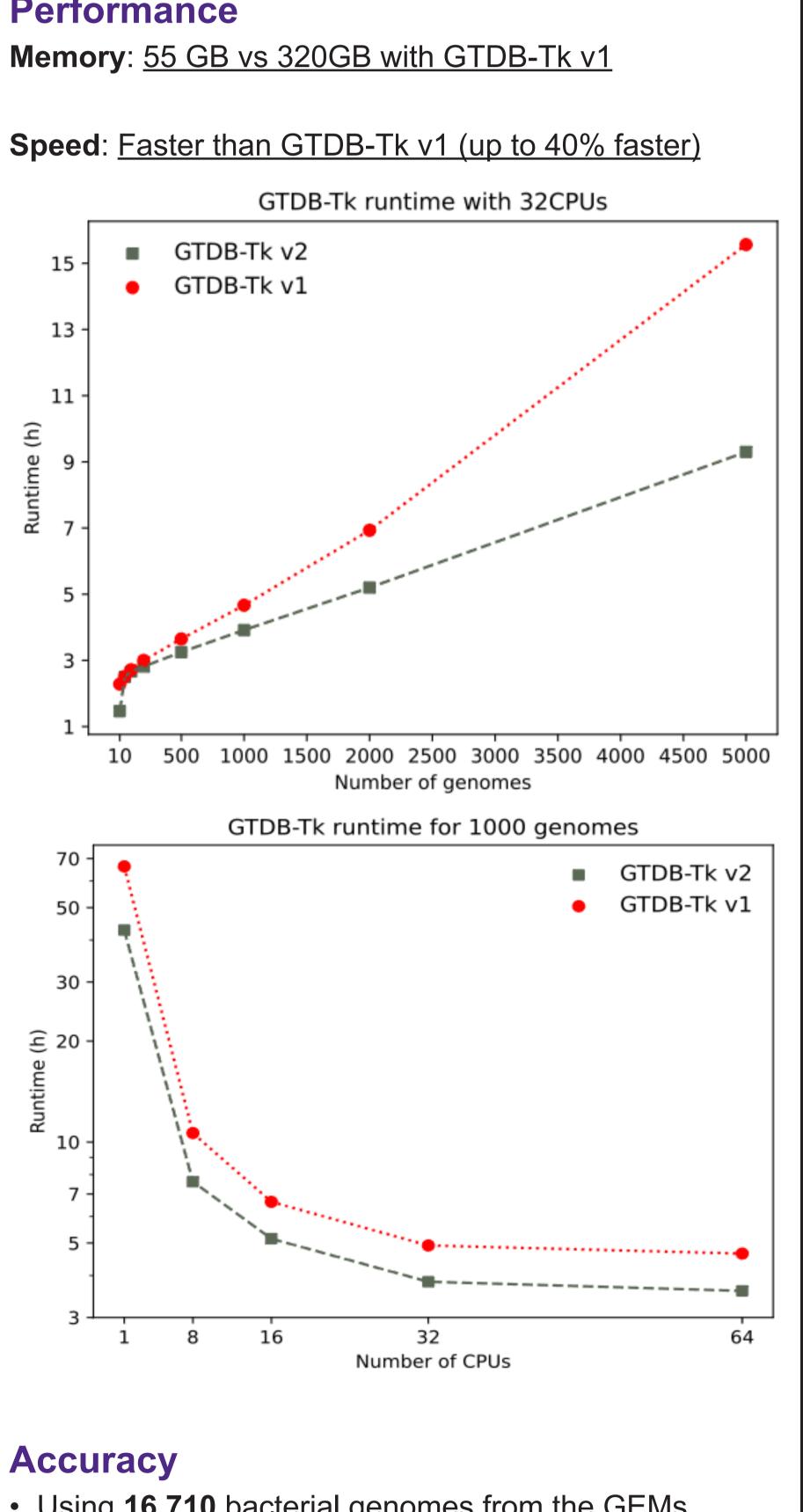
Query genome will be classified as either a novel basal *Escherichia* species or a novel genus in the family *Enterobacteriaceae* depending on its RED value.



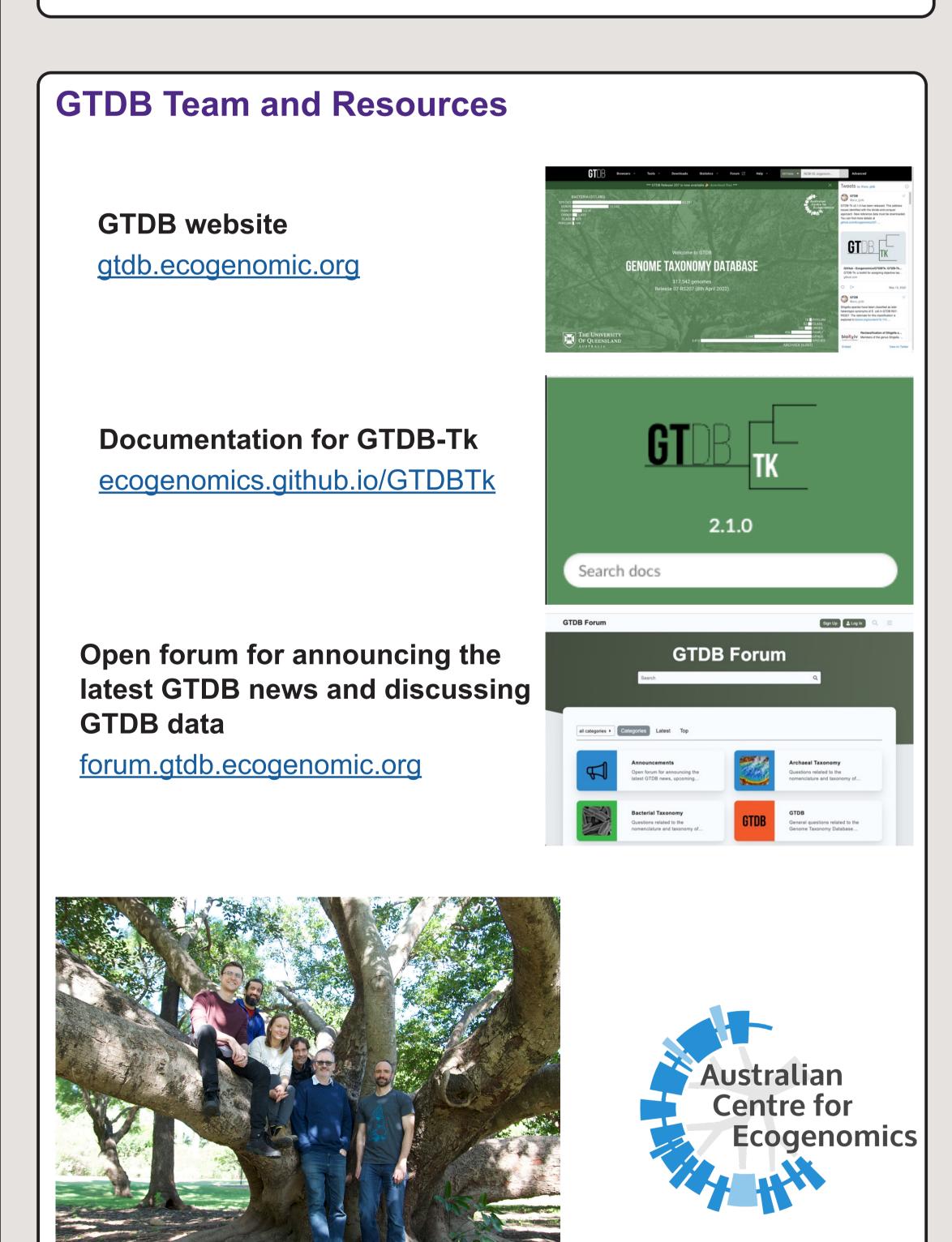
The query genome is assigned to the closest Staphylococcus species if the ANI is above the species ANI circumscription radius or is otherwise classified as a novel species.



Aerophobia is the only class within the Aerophobota phylum and as such, the query genome may be classified as the most basal order in Aerophobia, a novel class within the Aerophobota, or a novel phylum depending on its RED value.



- Using **16,710** bacterial genomes from the GEMs dataset (*Nayfach et al., 2021*), **12** genomes (0.07%) did not have identical classifications between GTDB-Tk v1 and GTDB-Tk v2 (**See Table**)
- Using **23,548** genomes introduced in GTDB R07-RS207 classified using GTDB-Tk R06-RS202, **13**



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GTDB-Tk v2 preprint