

Re-Defining *Clostridioides difficile* Using Global Phylogenomic Analyses

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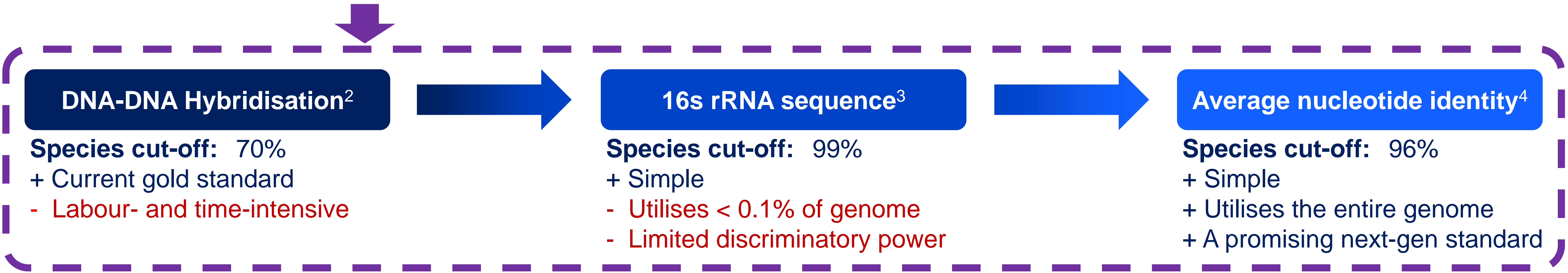
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Background and Objectives

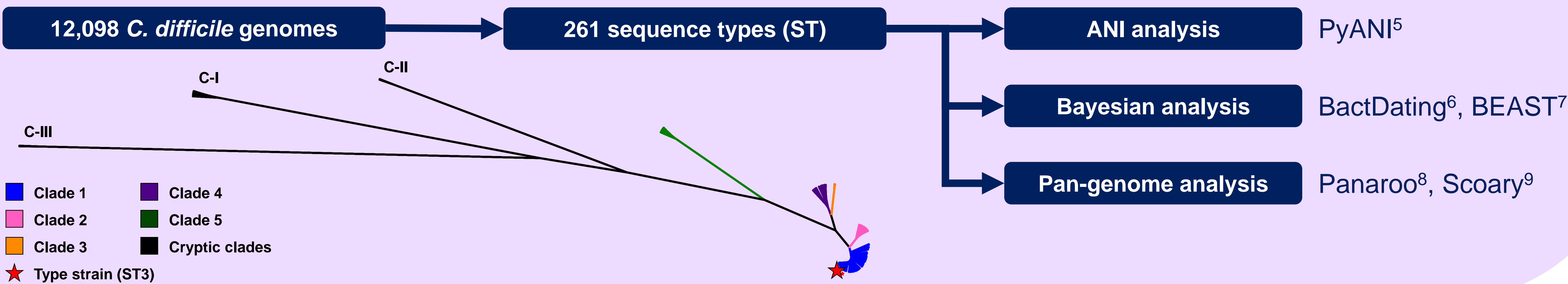
Bacterial species¹ = unique genotype + unique phenotypes (two or more independent phenotypes)



Clostridioides difficile species problem: A diverse population classified into a single species based on 16s rRNA

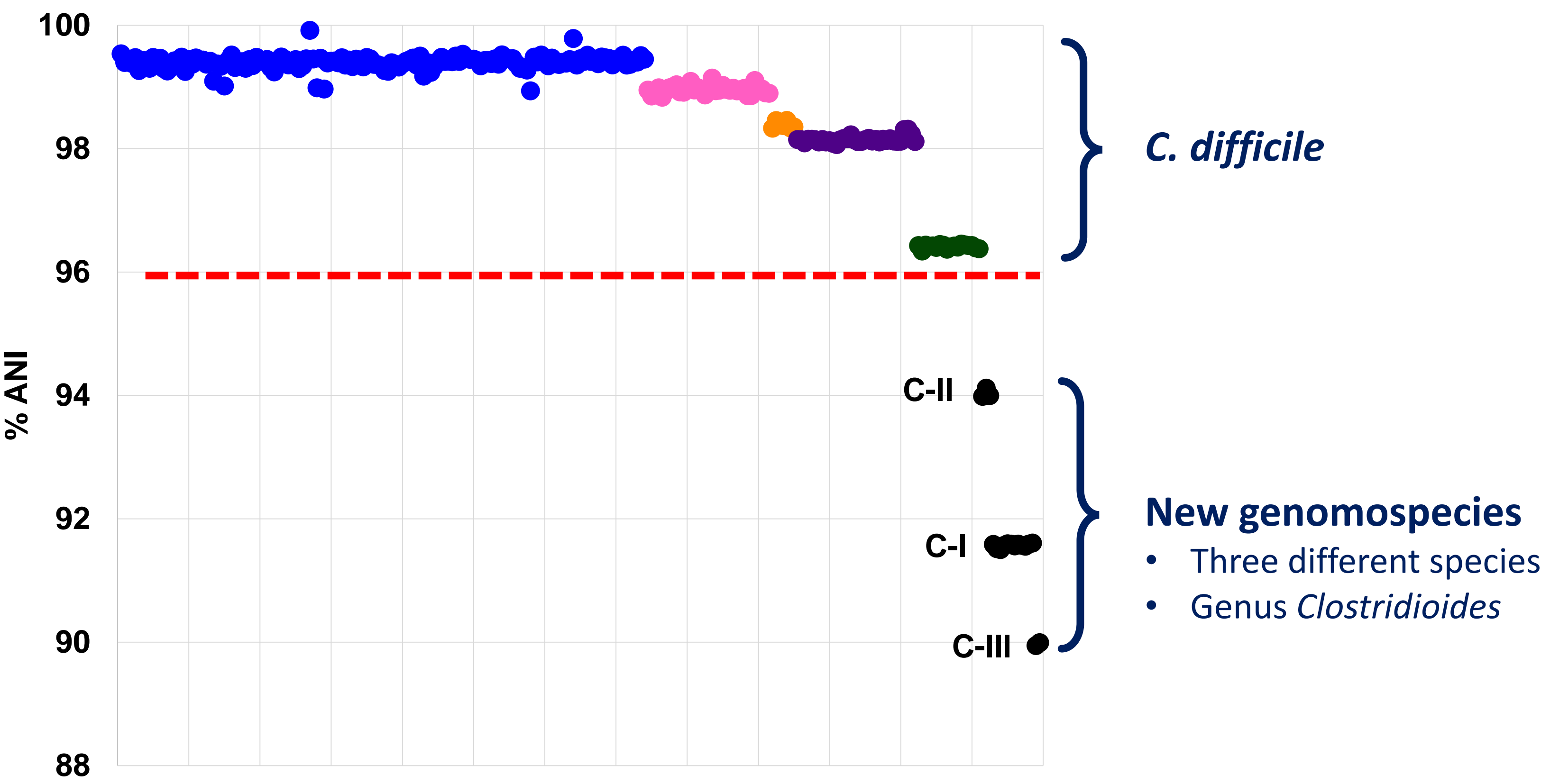
Study objective: Use ANI to re-evaluate the species definition of *C. difficile*

Methods

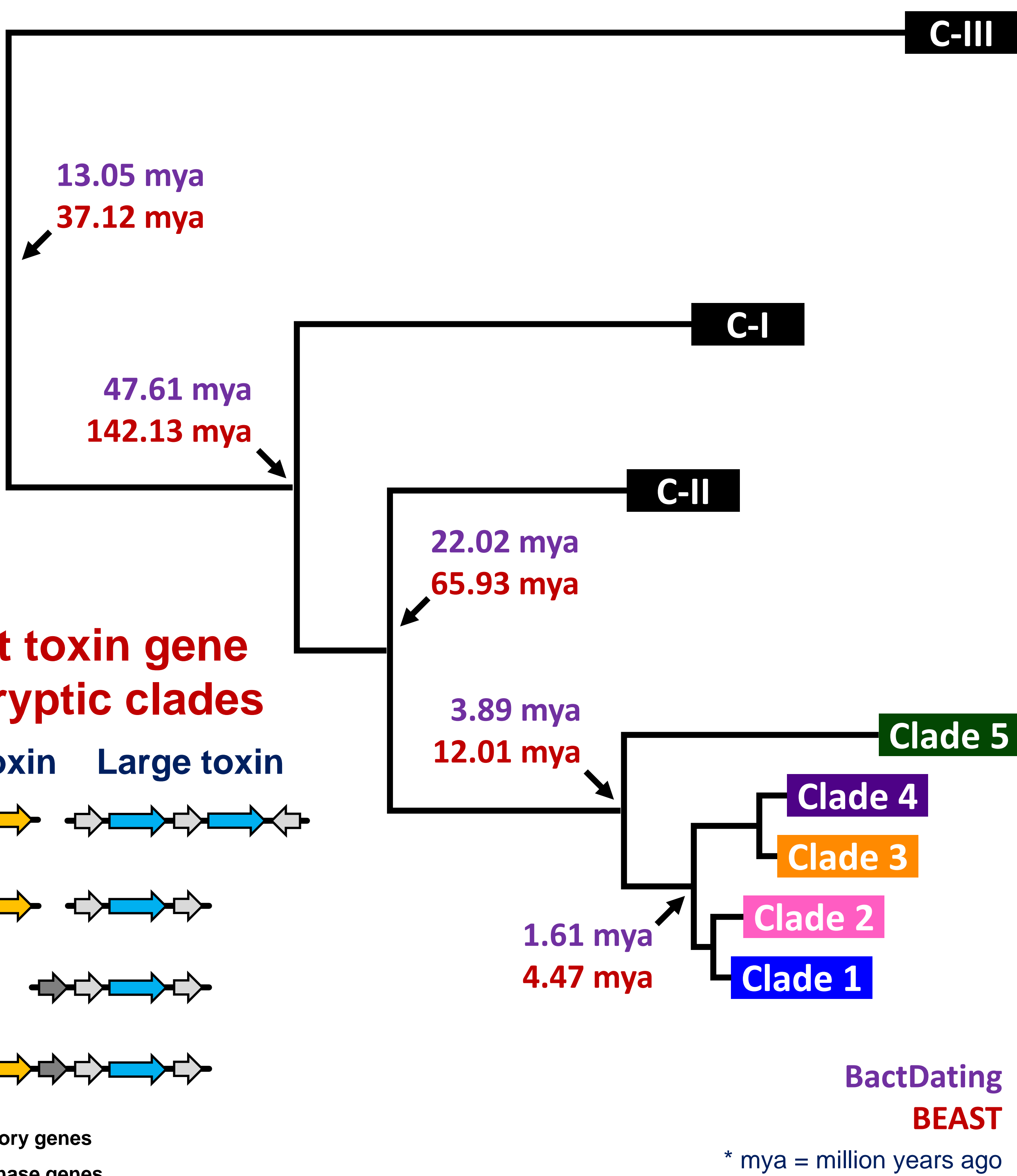


Results

ANI analysis reveals major discontinuity in *C. difficile* taxonomy



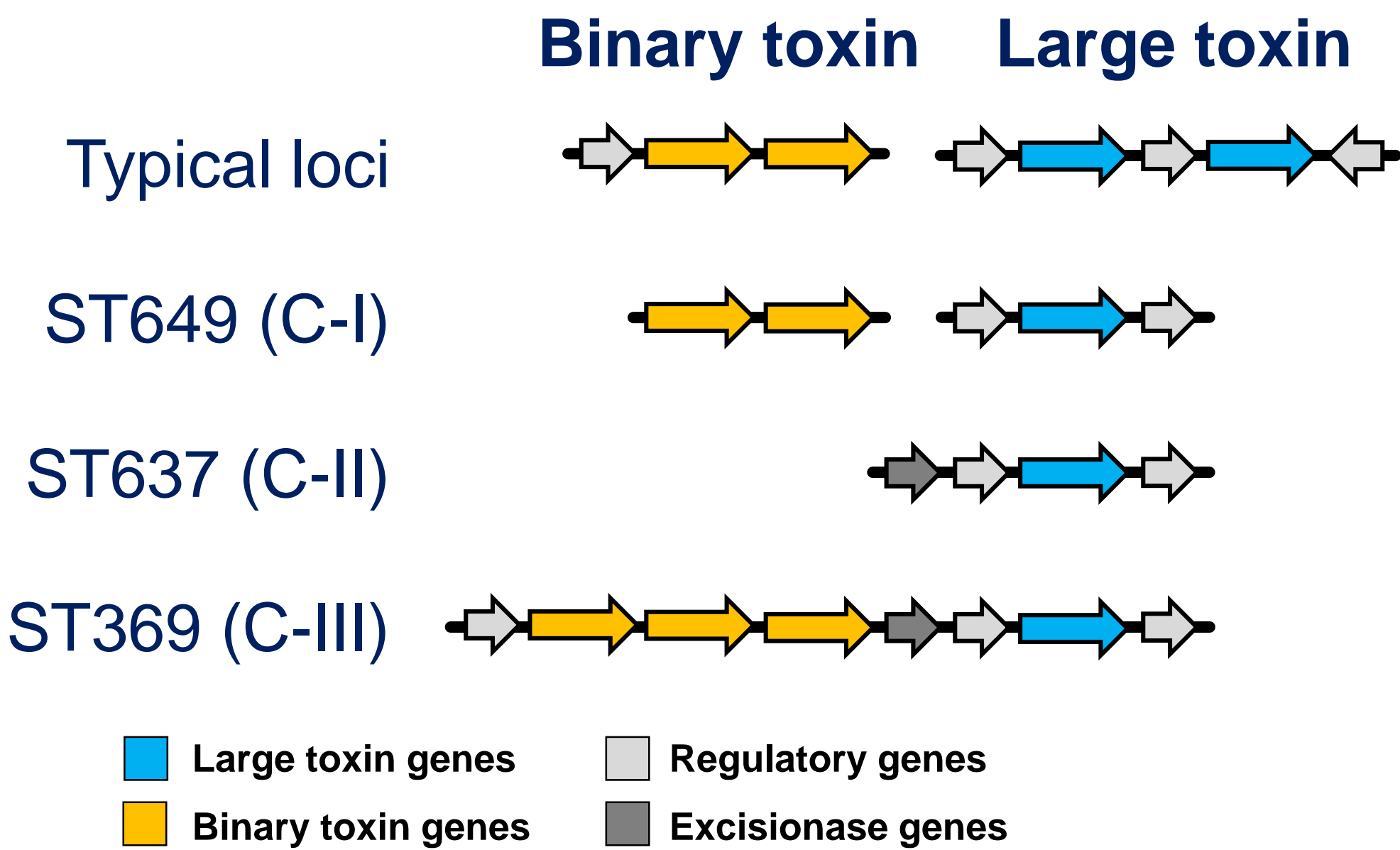
Bayesian evolutionary analysis reveals cryptic clades are ancient species



Pan-genome analysis identifies clade-specific genes and traits

Clades 1 – 4 14,869 genes	Total gene repertoire 17,470 genes
Clade 5 + 519 genes	Core gene 2,232 genes (12.8%)
Cryptic clades + 2,082 genes	Potential specific traits
	C-I - Fructosamine utilisation
	C-II - EDTA resistance
	C-III - Ethanolamine catabolism
	- Polyamine biosynthesis

Highly divergent toxin gene architecture in cryptic clades



Conclusions

There was a clear species boundary separating *C. difficile* from the 3 novel genomospecies. Several potential phenotypic differences were identified. Difference in toxin gene architecture and its divergence may complicate the diagnosis of *C. difficile* infection.

References

- Konstantinidis KT, et al. (2006) *Philos Trans R Soc Lond B Biol Sci*;361:1929-1940.
- Wayne LG, et al. (1987) *Int J Syst Evol Microbiol*;37:463-464.
- Stackebrandt E, et al. (1994) *Int J Syst Evol Microbiol*;44:846-849
- Jain C, et al. (2018) *Nat Commun*;9:5114.
- Pritchard L, et al. (2016) *Anal Methods*;8:12-24.
- Didelot X, et al. (2018) *Nucleic Acids Res*;46:e134-e134.
- Drummond AJ, et al. (2007) *BMC Evol Biol*;7:214.
- Tonkin-Hill G, et al. (2020) *Genome Biol*;21:180.
- Brynildsrud O, et al. (2016) *Genome Biol*;17:238.

