

Species-Wide Genetic Atlas of Antimicrobial Resistance in *Clostridioides difficile*

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Background and Objectives

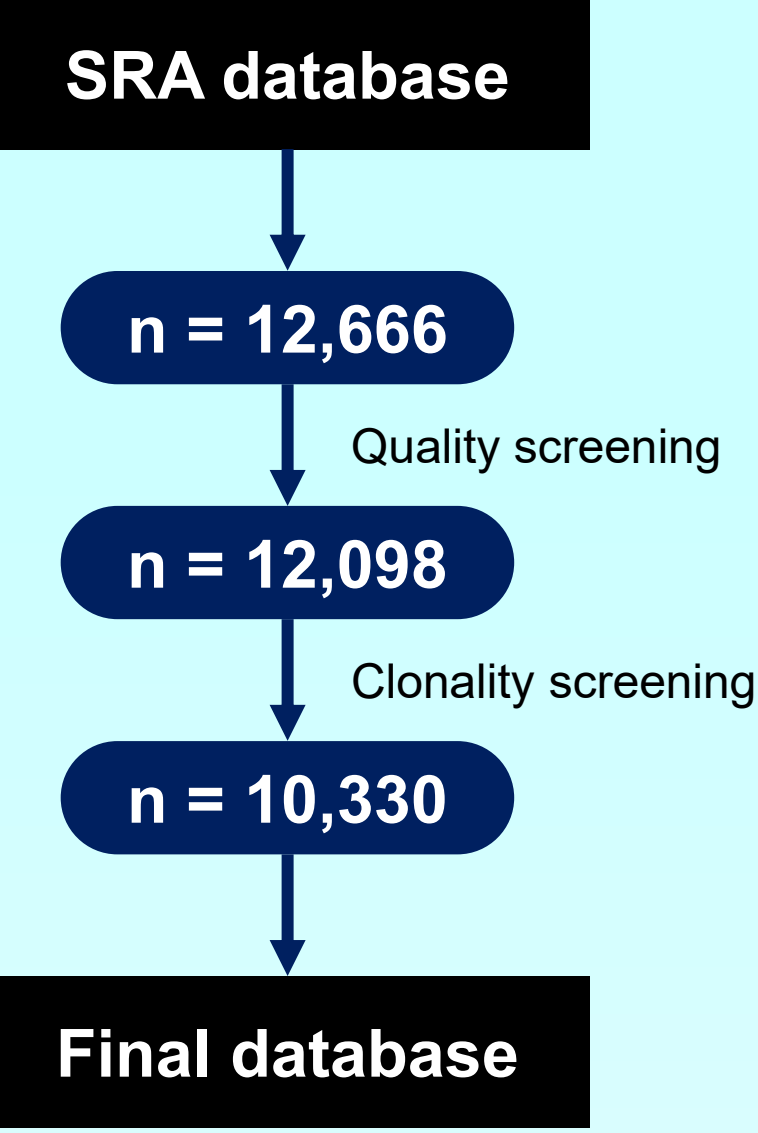
Antimicrobial resistance (AMR) plays an important role in the spread and pathogenesis of *Clostridioides difficile* infection (CDI). An association between AMR and CDI outbreaks has been identified^{1,2,3}, however, such studies have been limited to a few strains in limited geographical regions.

This study aimed to investigate the prevalence of AMR genotypes in the global population of *C. difficile*.

Methods

- All publicly available read files were downloaded.
- All non-redundant illumina read files were screen for quality and contamination using kraken2⁴.
- Read files of epidemic strains (sequence types (STs) 1, 2, 11 and 37) were screened for clonality (nucleotide identity > 99.98%) using SRST2⁵ and Sketch⁶, respectively.
- All remaining read files were interrogated against several AMR databases.[#]

Sample size



Gene databases[#]

Gene alleles

- MLST alleles⁷
- *gyrA*/*gyrB*⁷ (Fluoroquinolones)
- *rpoB*⁷(Rifamycins)
- *pbp1* & *pbp3*^{*}(Carbapenems)

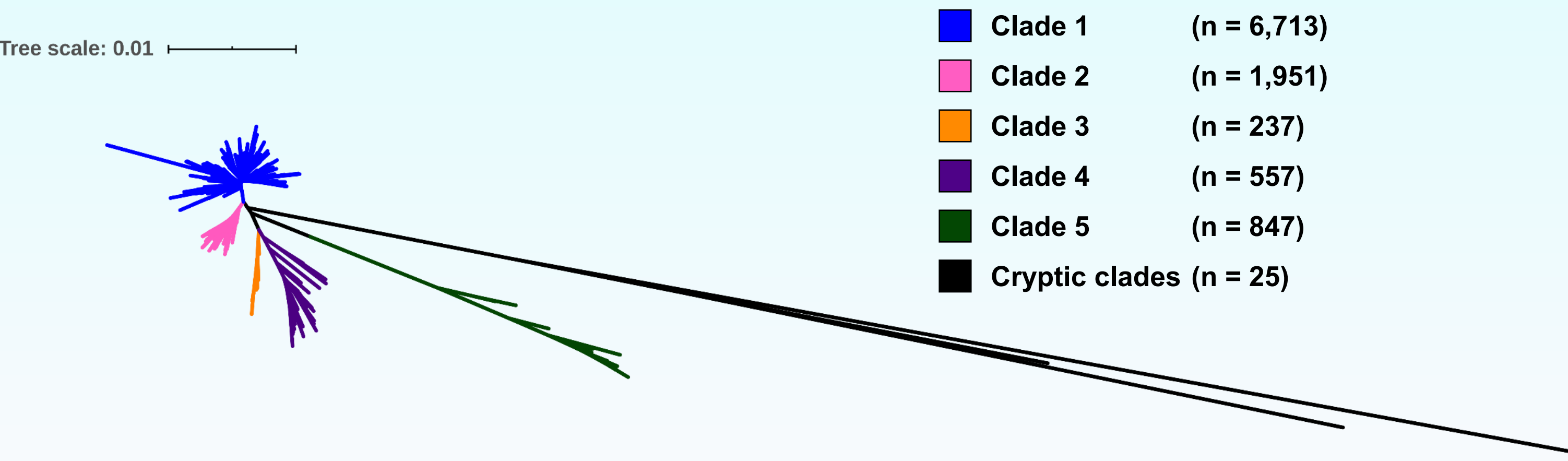
Accessory genes

- ARGannot⁸
- Resfinder⁹
- ^{*} customised databases

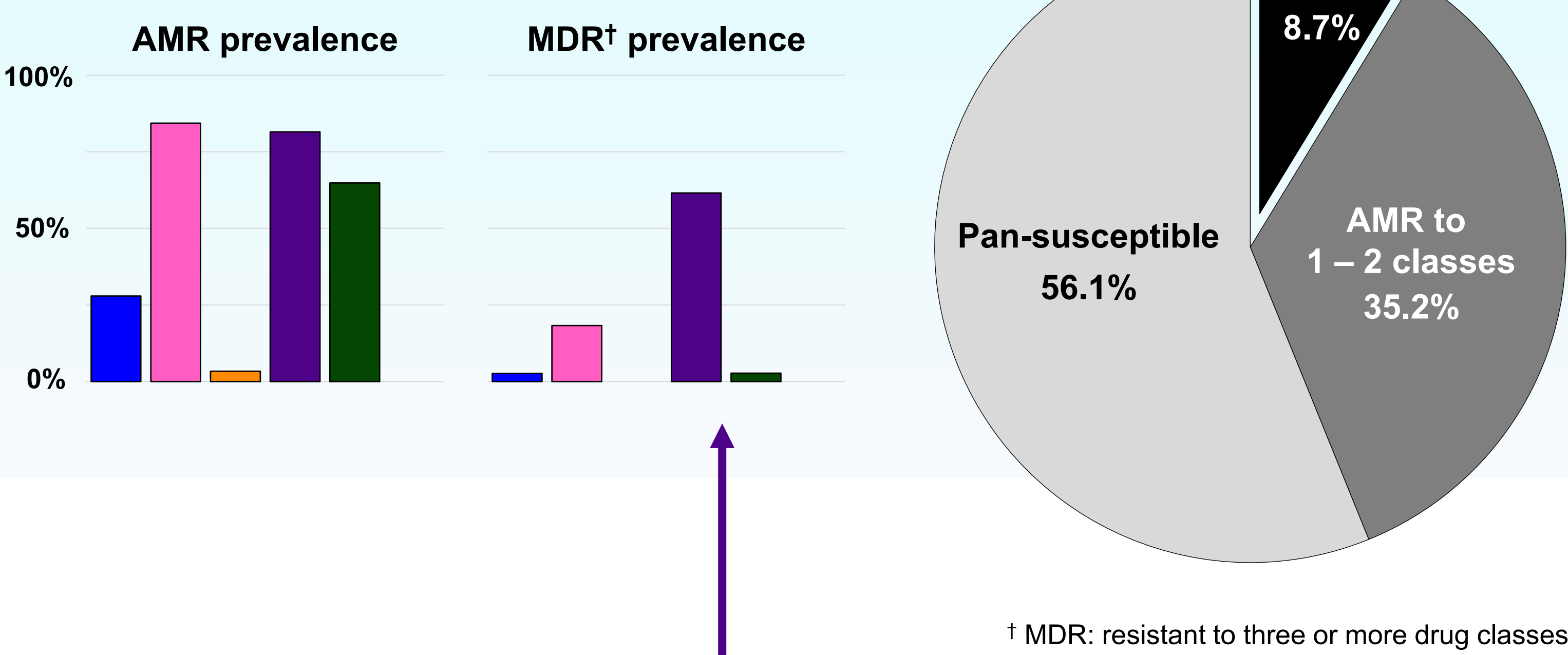
Detailed methods available here **QR**

Results

1. Population structure of *C. difficile*



2. Overall AMR prevalence



Epidemic *C. difficile* STs

ST1 (clade 2)

Affected regions: North America & Europe

Associated drug classes: Fluoroquinolones & Rifamycins

ST11 (clade 5)

Affected regions: Europe

Associated drug classes: Tetracyclines

ST37 (clade 4)

Affected regions: Global

Associated drug classes: MLS_B, Tetracyclines & MDR

Resistance prevalence by drug classes

Fluoroquinolones

common determinants	T82I (GyrA)	D426N (GyrB)
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Rifamycins

	R505K (RpoB)	H502N (RpoB)
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Carbapenems

	A555T (PBP1)	Y721S (PBP2)
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Tetracyclines

	<i>tetM</i>	<i>tet40</i>
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MLS_B

	<i>ermB</i>	<i>erm(52)</i>
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Aminoglycosides

	<i>aac6-aph2</i>	<i>aph-III, sat4</i> cluster
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Point substitutions

Accessory genes

Conclusion

There was a higher prevalence of AMR among epidemic *C. difficile* lineages. Despite its intrinsic resistance, some *C. difficile* strains carried aminoglycoside resistance genes, suggesting its role as a reservoir of AMR genes.



References

- Dingle KE, *et al.* (2019). *MBio*:10(2):e02790-18.
- He M, *et al.* (2013). *Nat Gen*; 45(1):109-13.
- Imwattana K, *et al.* (2020). *Expert Rev Anti Infect Ther*;1:17-25.
- Wood DE, *et al.* (2019). *Genome Biol*;20(1):257.
- Inouye M, *et al.* (2014). *Genome Med*;6(11):90.
- BBtools package [available at <https://sourceforge.net/projects/bbmap/>]
- PubMLST database [available at <https://pubmlst.org/cdifficile/>]
- Gubta SK, *et al.* (2014). *Antimicrob Agents Chemother*;58(1):212-20.
- Zankari E, *et al.* (2012). *J Antimicrob Chemother*; 67(11):2640-4.

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Re-Defining *Clostridioides difficile* Using Global Phylogenomic Analyses

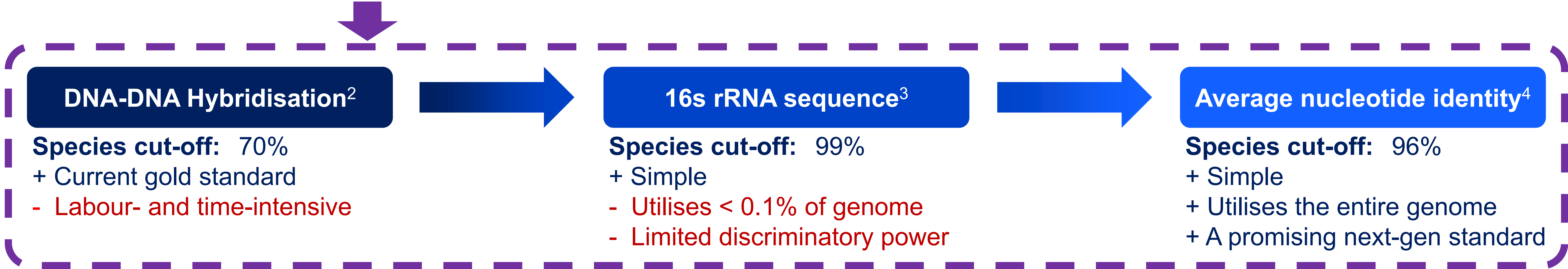
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Background and Objectives

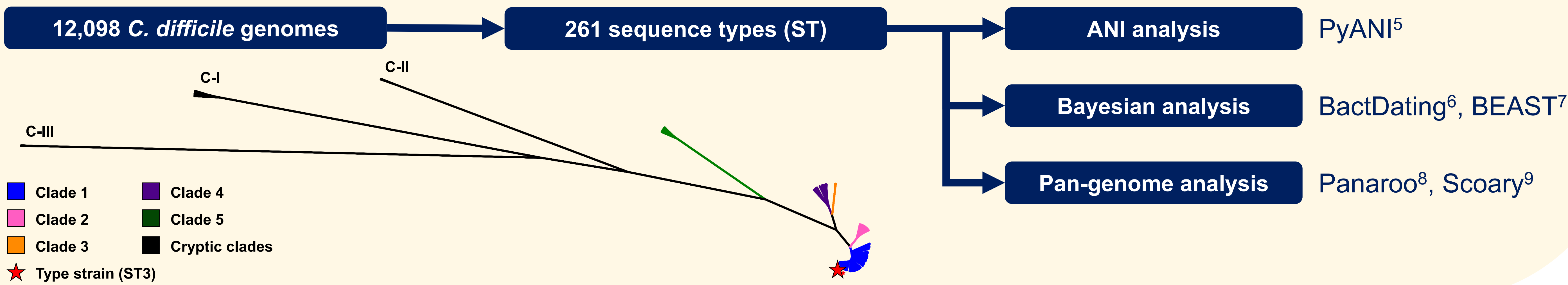
Bacterial species¹ = unique genotype + unique phenotypes (two or more independent phenotypes)



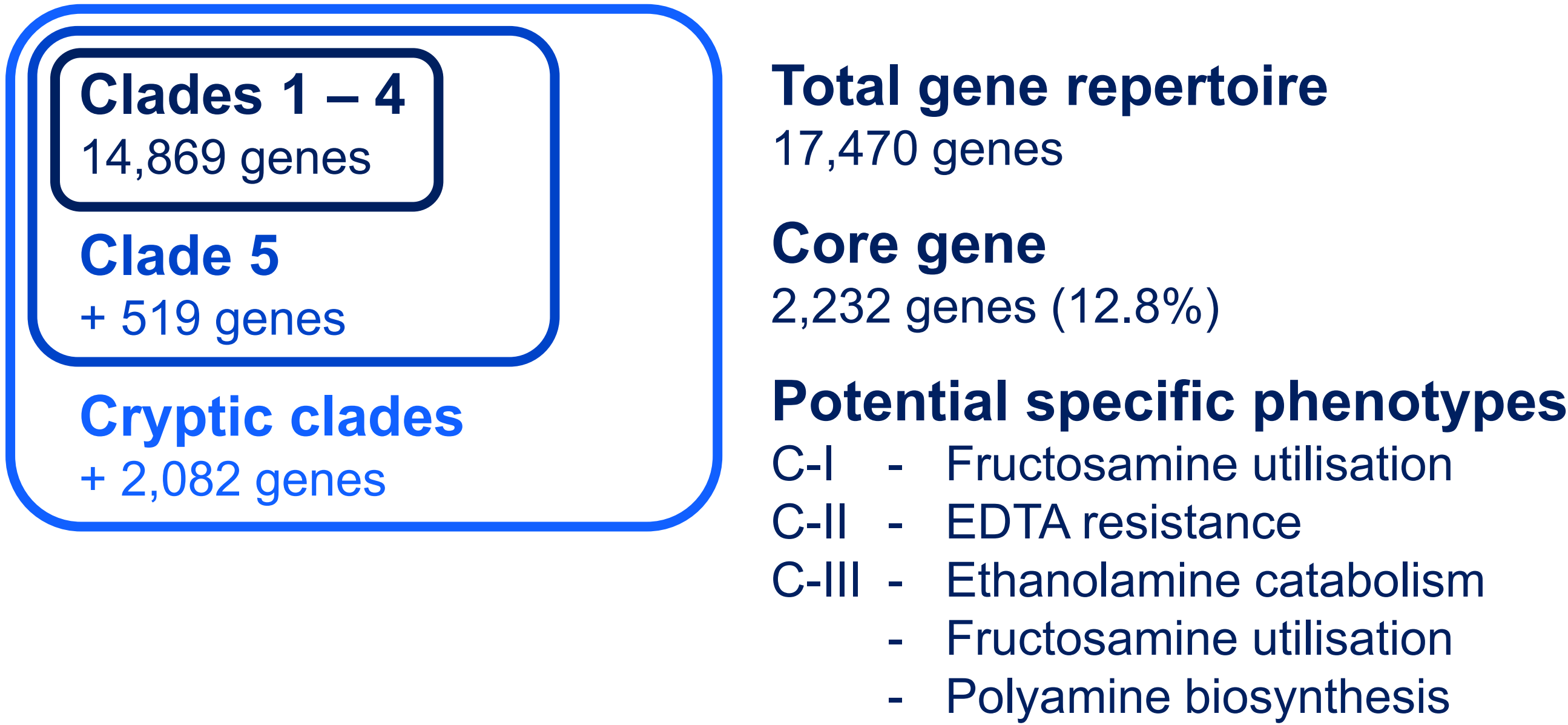
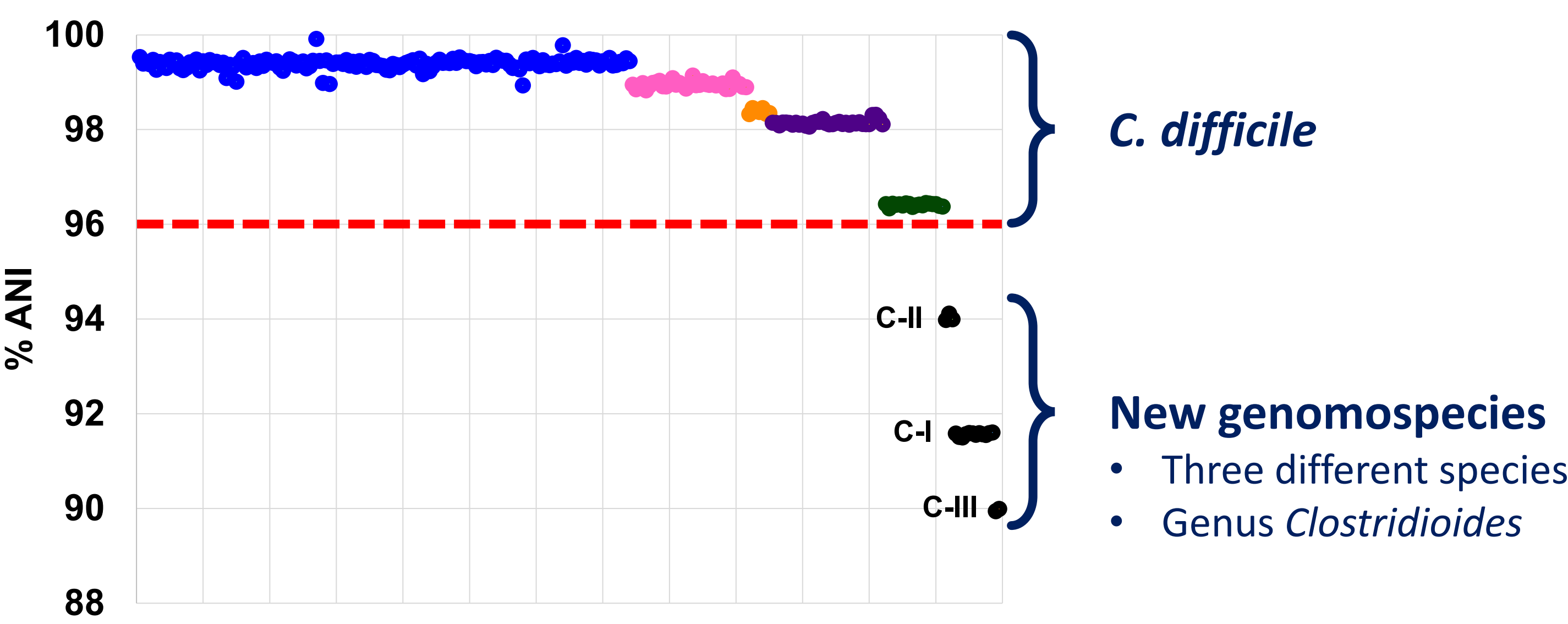
Clostridioides difficile species problem: A diverse population classified into a single species based on 16s rRNA

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

Methods

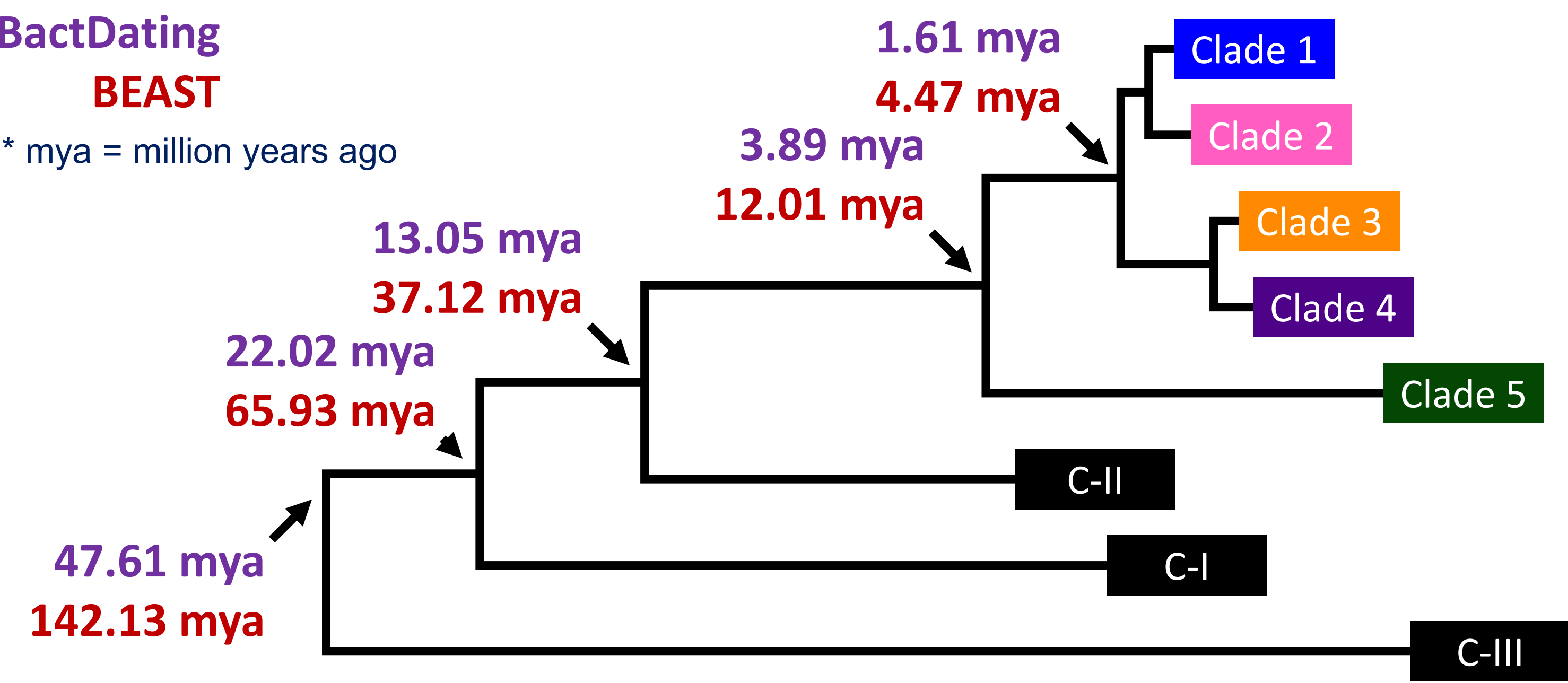


Results

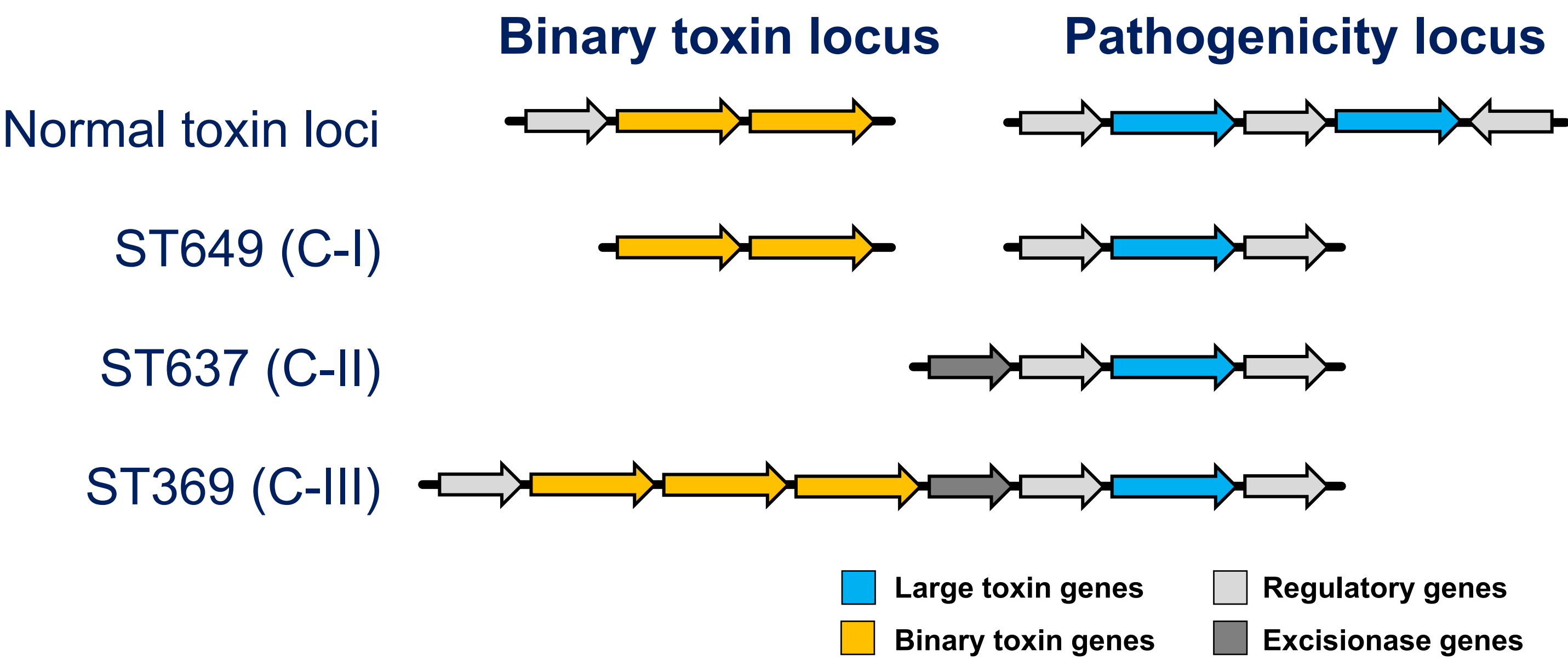


1. ANI analysis reveals major discontinuity in *C. difficile* taxonomy

3. Pan-genome analysis identifies clade specific genes and traits



2. Bayesian evolutionary analysis reveals cryptic clades are ancient species



4. Cryptic clades harbor novel and highly divergent toxin gene architecture identifies

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

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