



SCAN ME

EMERGENCE OF NEW multi-resistant *Brachyspira hyodysenteriae* in Belgian pigs, revealed by high quality whole genome sequencing

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Introduction

Swine dysentery (SD) caused by *Brachyspira hyodysenteriae* (*B. hyodysenteriae*) results in substantial economic losses in swine-producing countries worldwide. Outbreaks of SD caused concern in Belgium, given the sudden rise of cases that were admitted to the laboratory Animal Health Care Flanders in 2018-2020. Furthermore, it was feared that treatment with antimicrobial compounds would be hampered due to an **increased resistance** against commonly used antibiotics. Therefore, an in-depth genomic analysis of antimicrobial resistance and genetic relatedness of Belgian strains was done in this study.

- various hosts (e.g. rodents, dogs, birds)
- *Spirochaetes* bacterium
- 27% GC content
- anaerobic growth (3-10 days)
- 100% tylosine resistance*

Brachyspira hyodysenteriae

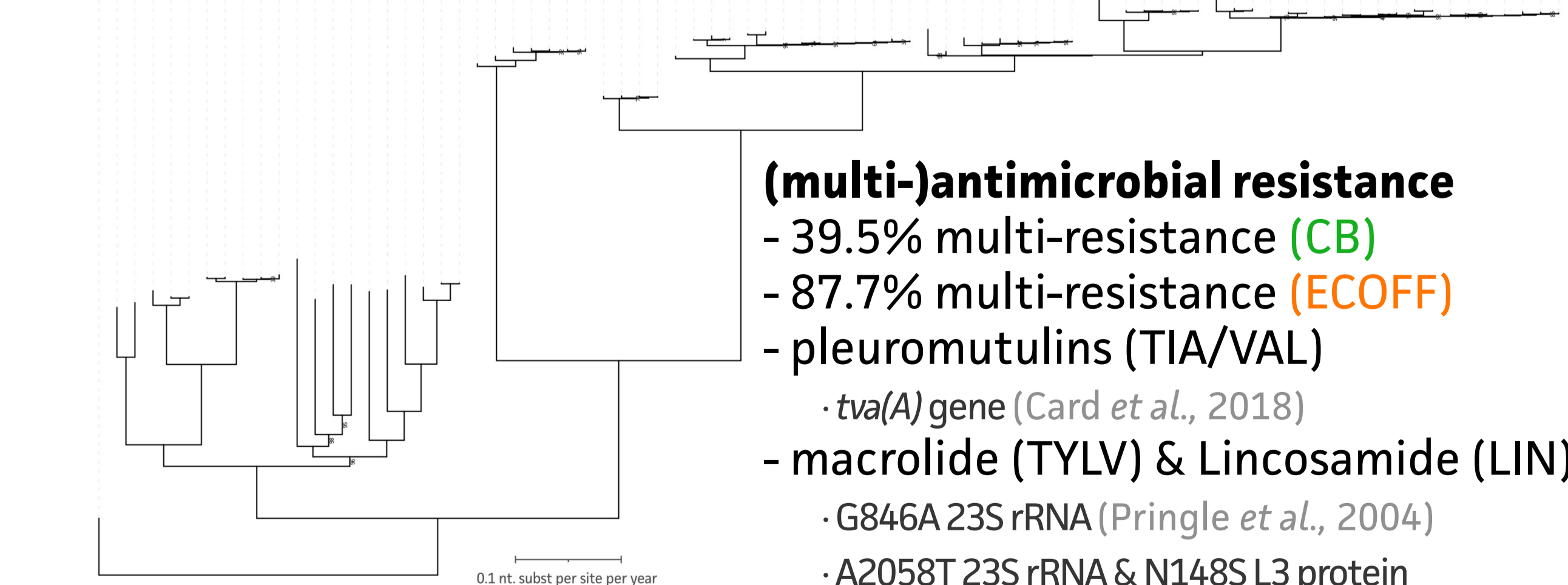
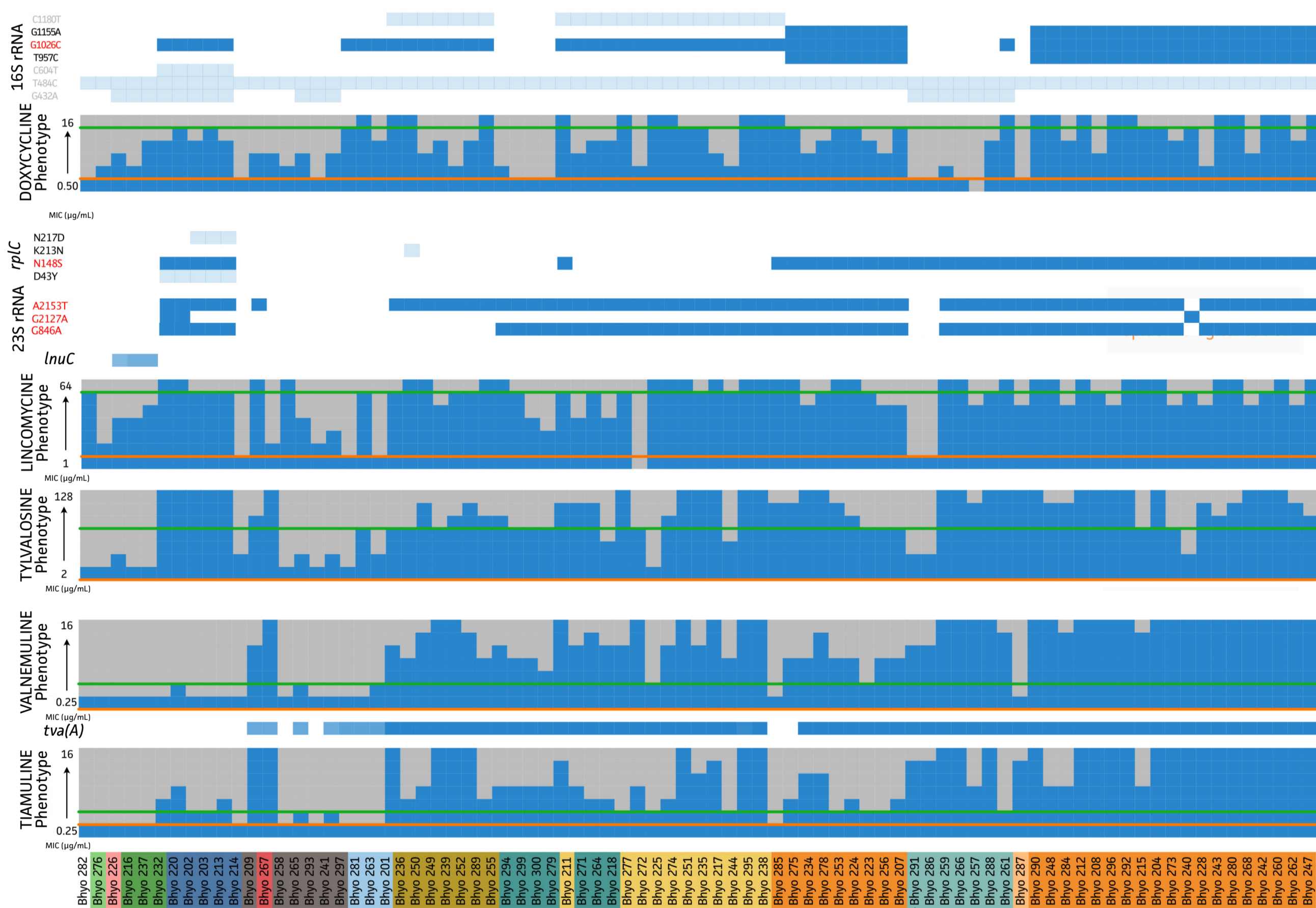
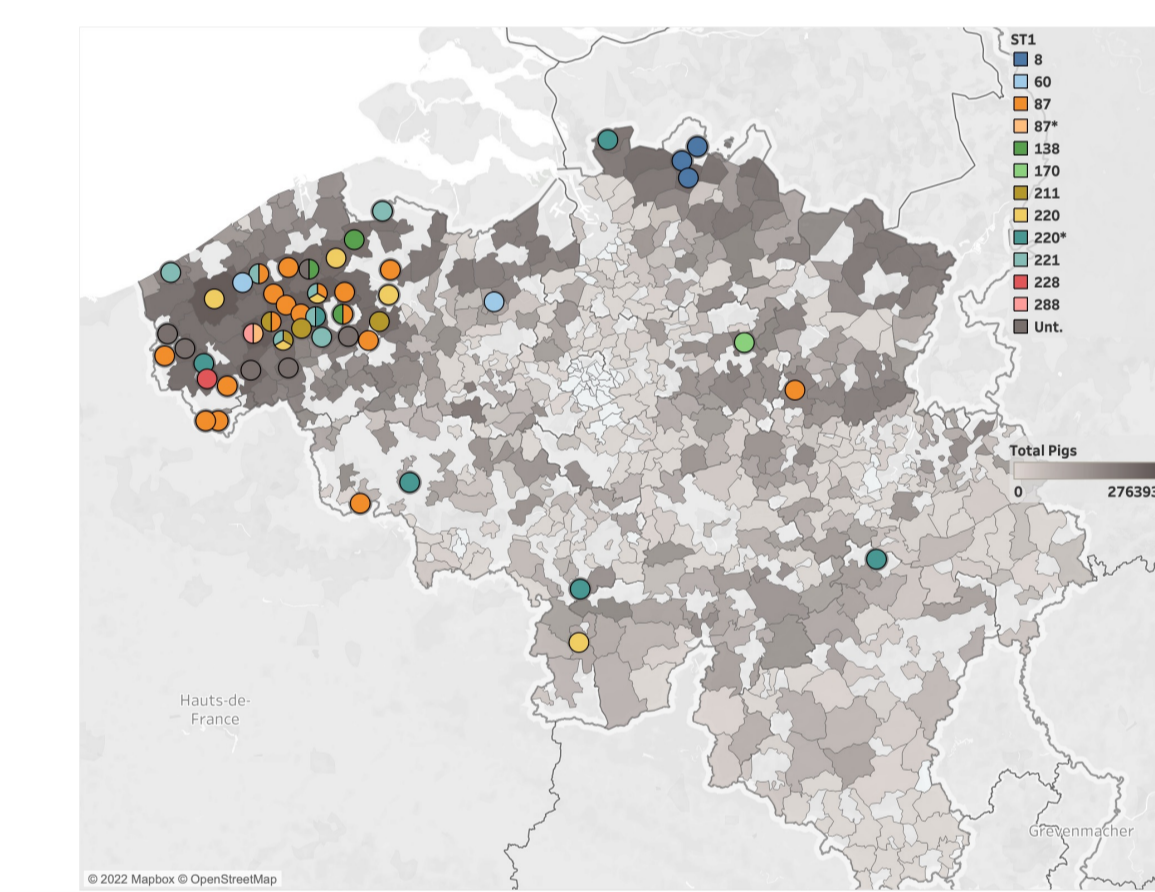
*AMCRA guidelines, Belgium 2022

Workflow

THE QUEST for markers

Multi-Locus Sequence Typing

- over 12 STs circulating since 2018
- ST87 34.6%
- ST220 13.6%
- 2 new STs (*ST87* and *ST220* derived)
- some **Untypable** strains (n=6)



(multi-)antimicrobial resistance

- 39.5% multi-resistance (CB)
- 87.7% multi-resistance (ECOFF)
- pleuromutulins (TIA/VAL)
 - *tva(A)* gene (Card *et al.*, 2018)
- macrolide (TYLV) & Lincosamide (LIN)
 - G846A 23S rRNA (Pringle *et al.*, 2004)
 - A2058T 23S rRNA & N148S L3 protein
 - no link *lnuC* gene
- tetracycline (DOXY)
 - G1026C 16S rRNA (Pringle *et al.*, 2007)
 - T957C & G1155A possible new 16S rRNA

clinical breakpoints epidemiological cut-off

Acknowledgements



90 *B. hyodysenteriae* field strains

- Belgian pigs (2018-2020)
- Belgian representation
- *B. hyodysenteriae* subcultures



AST phenotyping

- Agar dilution test
- **Antibiotics**
 - pleuromutulins (n=2)
 - macrolides (n=2)
 - lincosamides



Whole Genome Sequencing *

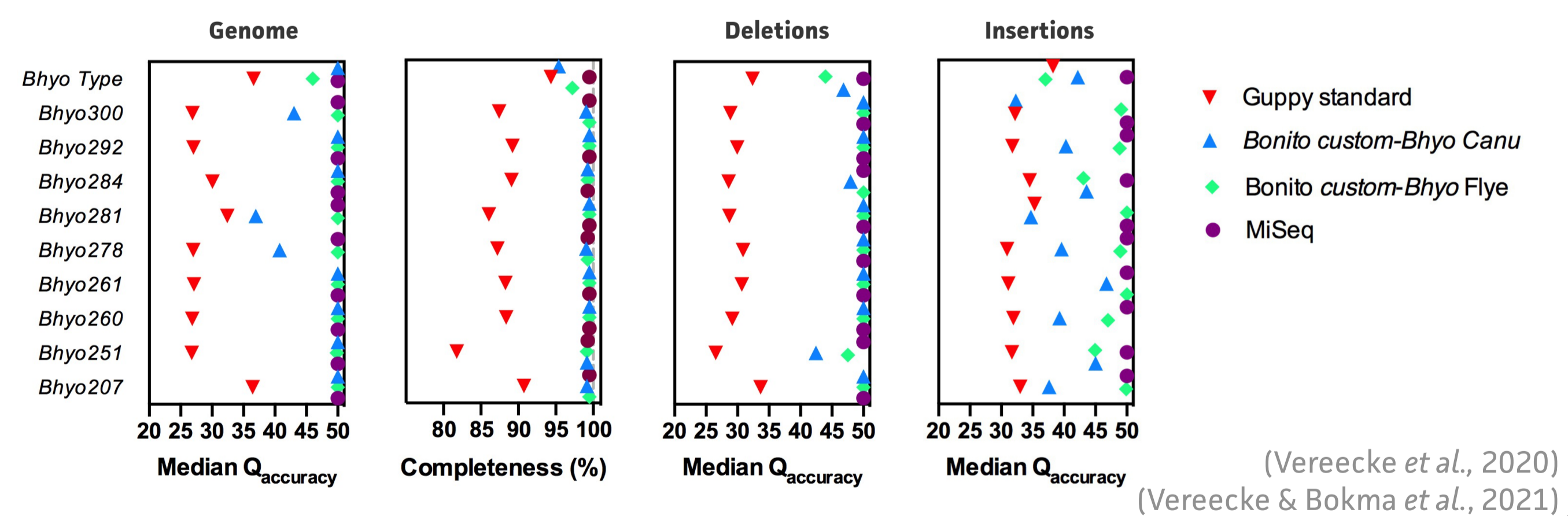
- MinION sequencer
- **Custom** Bonito (v.0.2.2)
- Flye (v.2.9) *de novo*
- Medaka (v1.0.0) polishing

Downstream

- MLST distribution
- WGS Phylogeny
- screening of
 - AMR mediators
 - PM (16S/23SrRNA)

* High Quality genomes with Nanopore reads?

- $Q_{Guppy} = 36 \pm 1 \rightarrow Q_{Bonito} = 51 \pm 2$
- average 23 errors in ± 3 Mbp *B. hyodysenteriae* genome



(Vereecke *et al.*, 2020)
(Vereecke & Bokma *et al.*, 2021)

Conclusions

- multiple (new) STs since 2018 with dominance of **ST87 & ST220**
- **ST8 & ST87** European origin before 2018 (Stubberfield *et al.*, 2021)
- high prevalence of **multi-resistant** *B. hyodysenteriae*
- multi-resistance **linked to MLST** (Untypable = low resistance)
- validation **ECOFFs Versus CBs** with WGS data
 - presence AMR genes and/or Point Mutations as **markers**
 - **potential new** (multi-)resistance markers (16S rRNA DOXY)
 - **re-evaluation** of current ECOFFs & CBs