ROSLIN

THE UNIVERSITY of EDINBURGH

Using genomic data to understand viral evolution and reconstruct transmission scenarios in Atlantic salmon aquaculture

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TriNation, Belfast

SAIC
Sustainable Aquaculture
Innovation Centre

PHARMAQ
part of zoetis

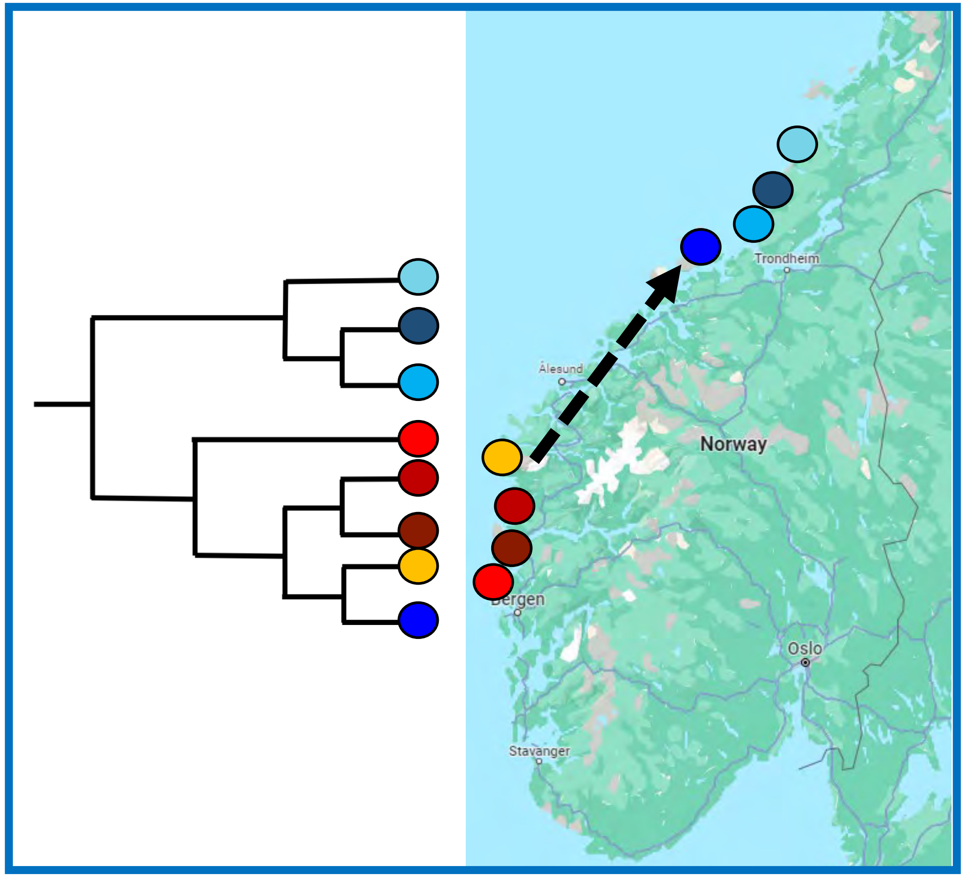
Whole genome sequencing

Project viruses:

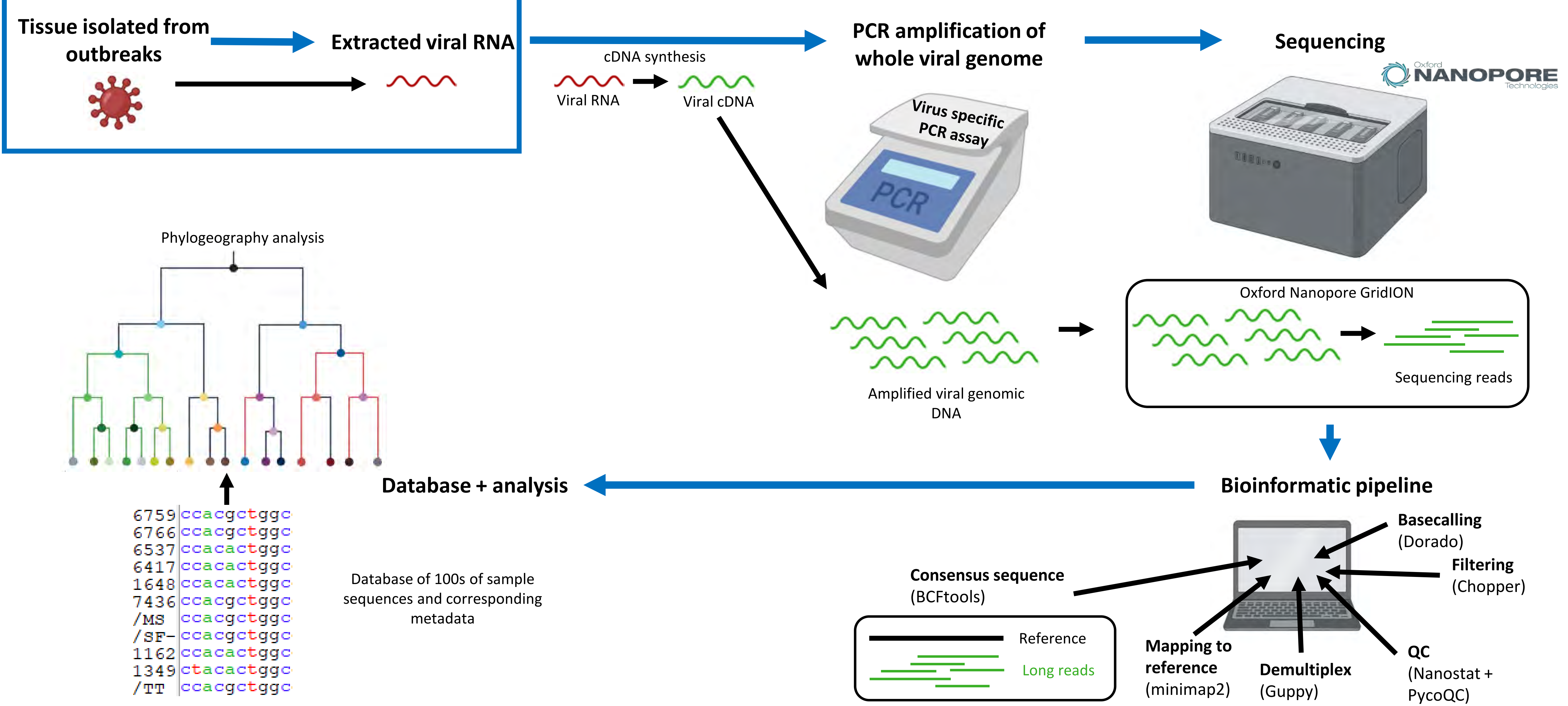
1. **Salmonid alphavirus (SAV)**
2. **Piscine Orthoreovirus (PRV)**
3. Infectious pancreatic necrosis virus (IPNV)

- Characterising viral genetic diversity
  - Understand spatial distribution of viral subtypes
  - Identify emergence of new viral subtypes

- Outbreak transmission routes
  - Map transmission of viral lineages
  - Aid infection control measures



Viral sequencing workflow



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Tissue isolated from outbreaks

Extracted viral RNA

cDNA synthesis

Viral RNA

Viral cDNA

PCR amplification of whole viral genome

Virus specific PCR assay

PCR

Sequencing

Oxford NANOPORE Technologies

Amplified viral genomic DNA

Oxford Nanopore GridION

Sequencing reads

Bioinformatic pipeline

Basecalling (Dorado)

Filtering (Chopper)

QC (Nanostat + PycoQC)

Demultiplex (Guppy)

Mapping to reference (minimap2)

Consensus sequence (BCFtools)

Reference

Long reads

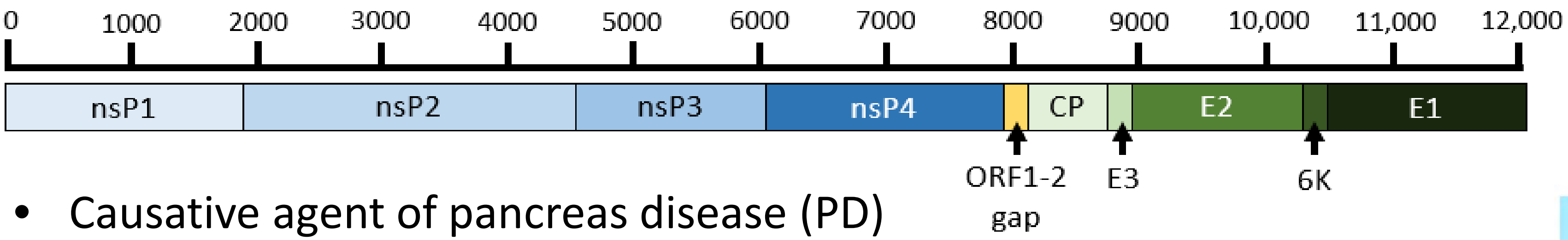
Database + analysis

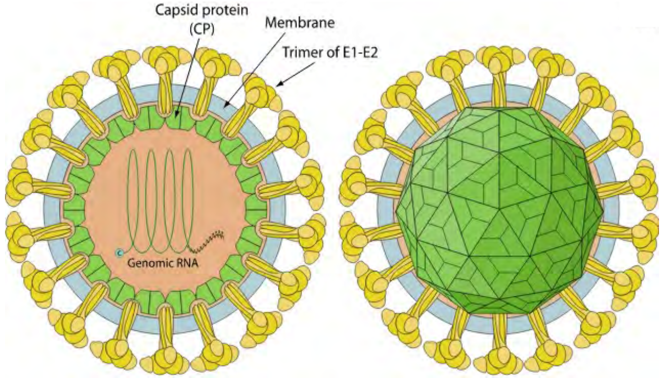
Phylogeography analysis

```
6759|ccacgctggc
6766|ccacgctggc
6537|ccacactggc
6417|ccacactggc
1648|ccacactggc
7436|ccacgctggc
/MS |ccacgctggc
/SF-|ccacgctggc
1162|ccacactggc
1349|ctacactggc
/TT |ccacgctggc
```

Database of 100s of sample sequences and corresponding metadata

Salmonid alphavirus (SAV)



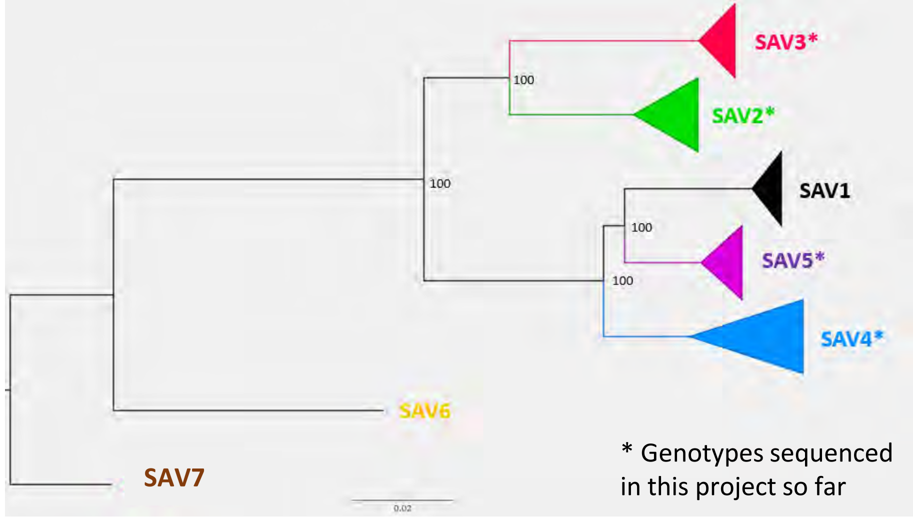


https://viralzone.expasy.org/625?outline=all_by_species

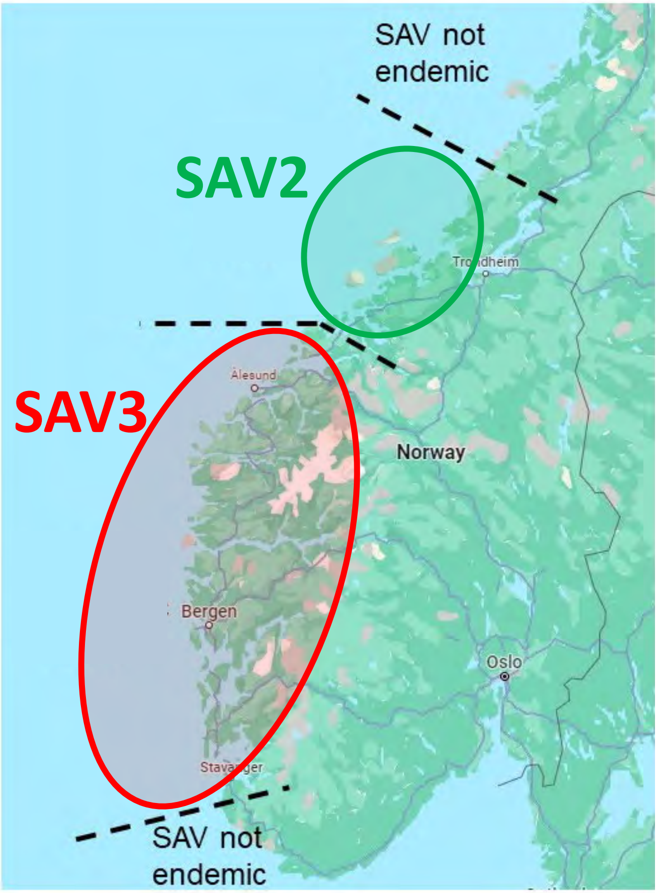
- Causative agent of pancreas disease (PD)
- ~12kb linear +ssRNA genome
- Horizontal transmission
- Seven genotypes: SAV1-7

SAV2 endemic zone:
Møre og Romsdal to Trøndelag

SAV3 endemic zone:
Rogaland to Sogn og Fjordane



* Genotypes sequenced in this project so far



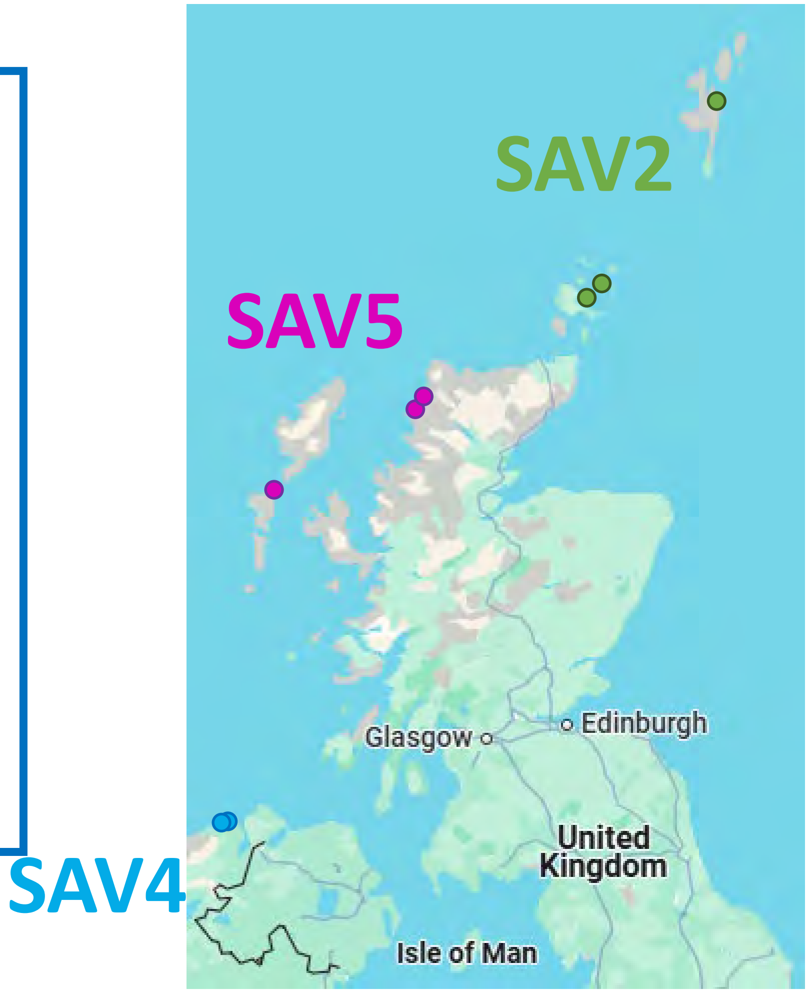
Producer locations (SAV)

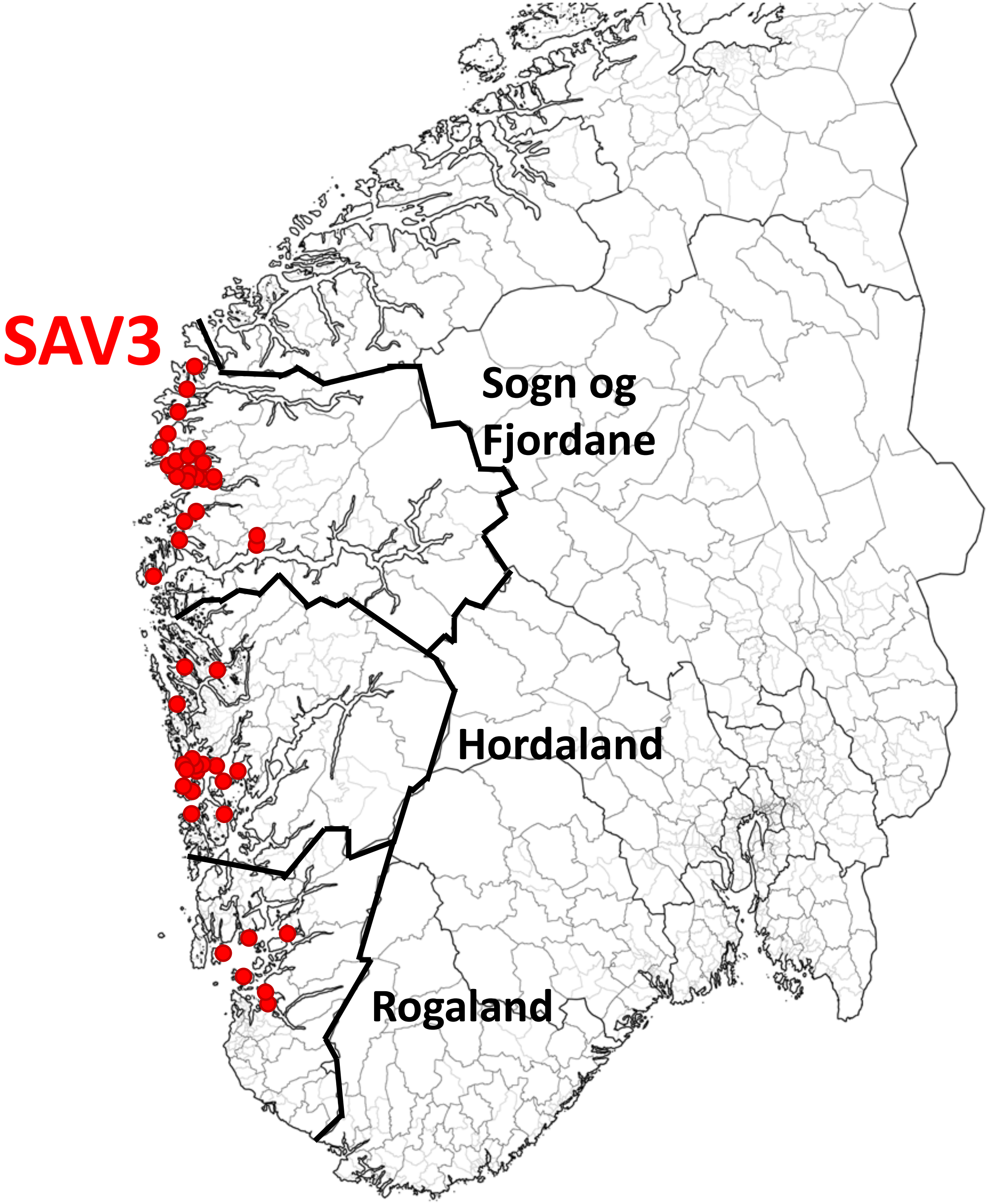
No. of samples: 189
- Norway: 171
- Scotland: 16
- Ireland: 2

No. of producers: 32
- No. of sites: 50

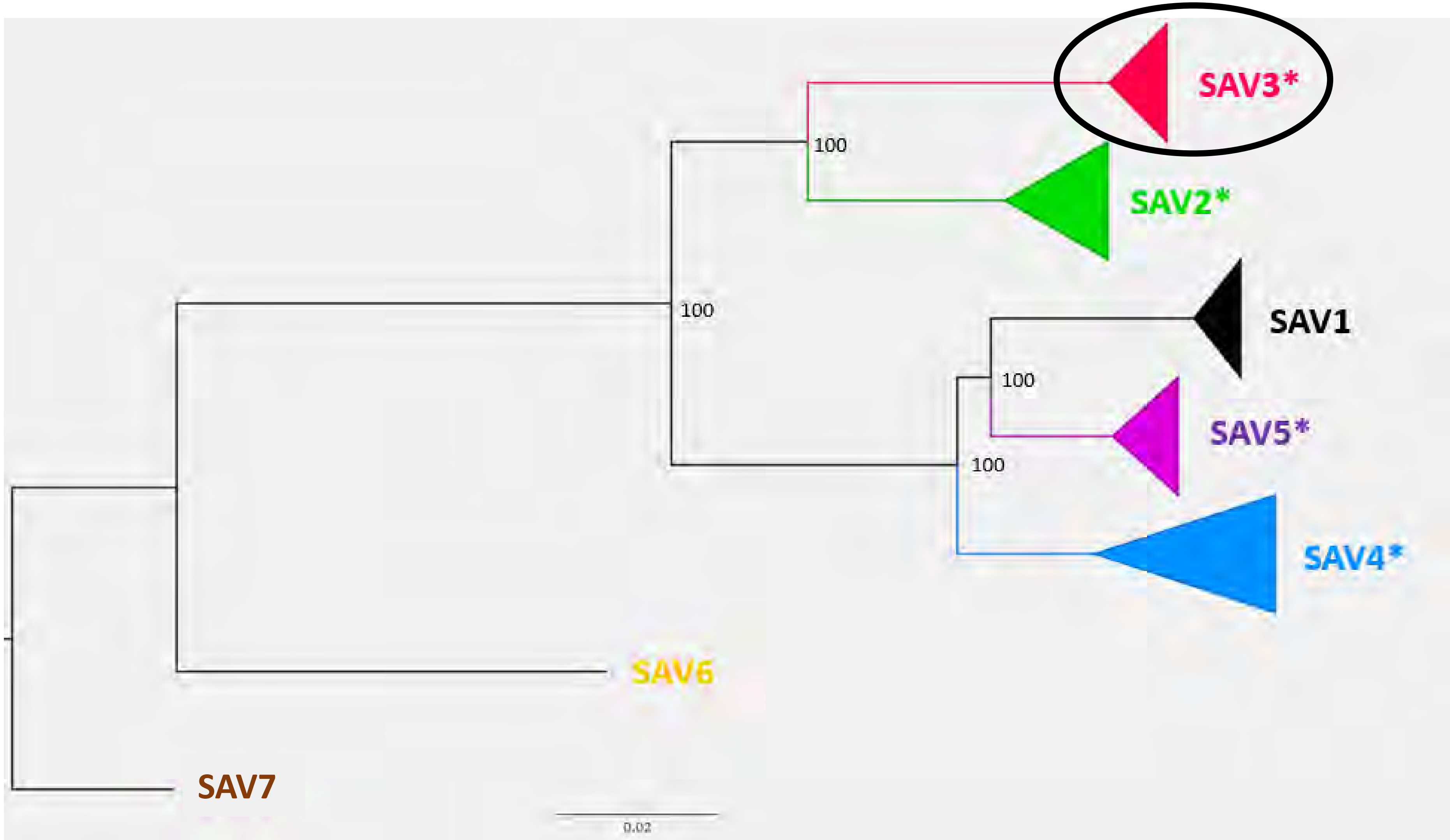
No. of outbreaks: 64

Date range:
July 2021 – May 2025





SAV3 in Norway

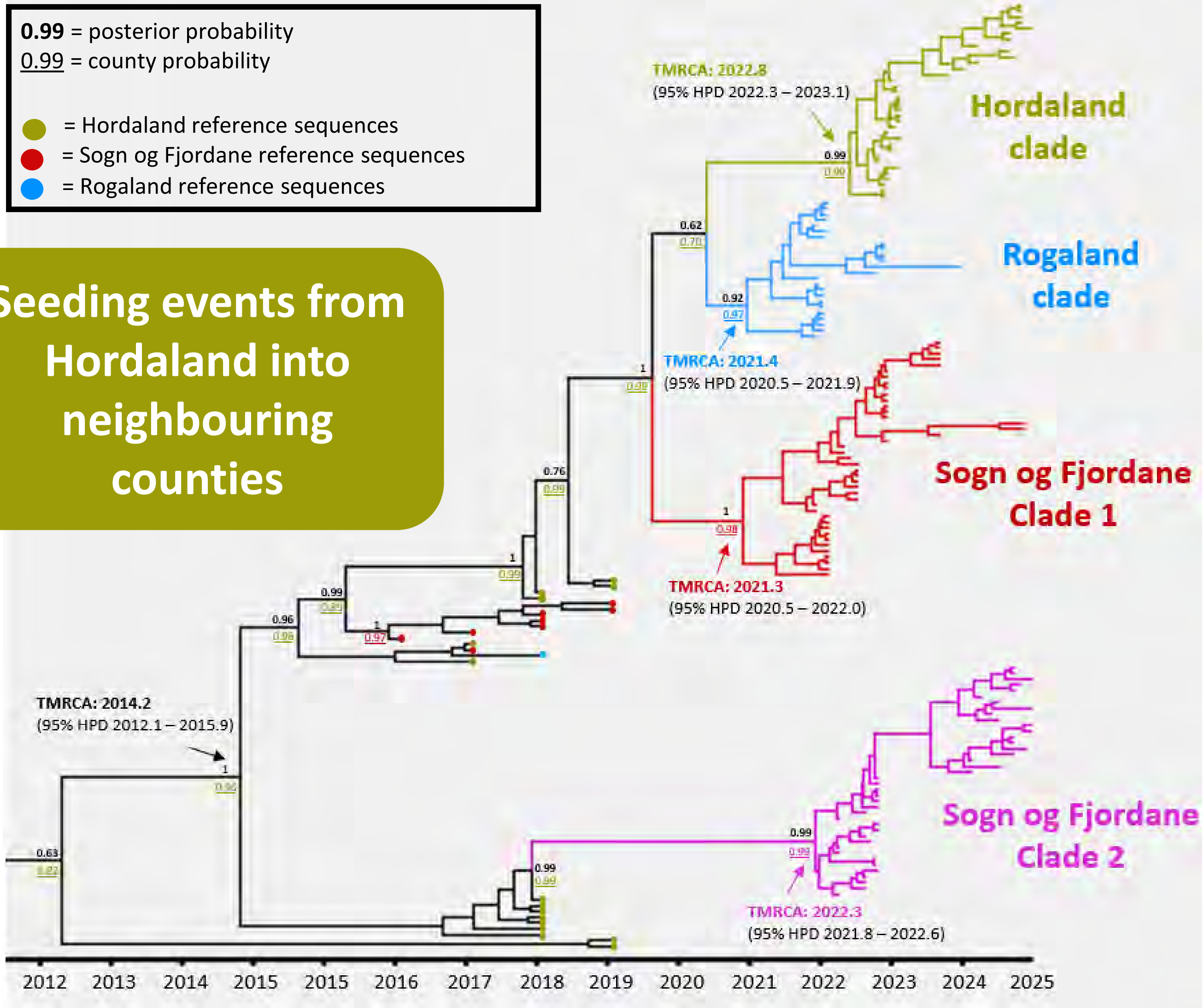


SAV3 in Norway

Discrete trait analysis (county data)

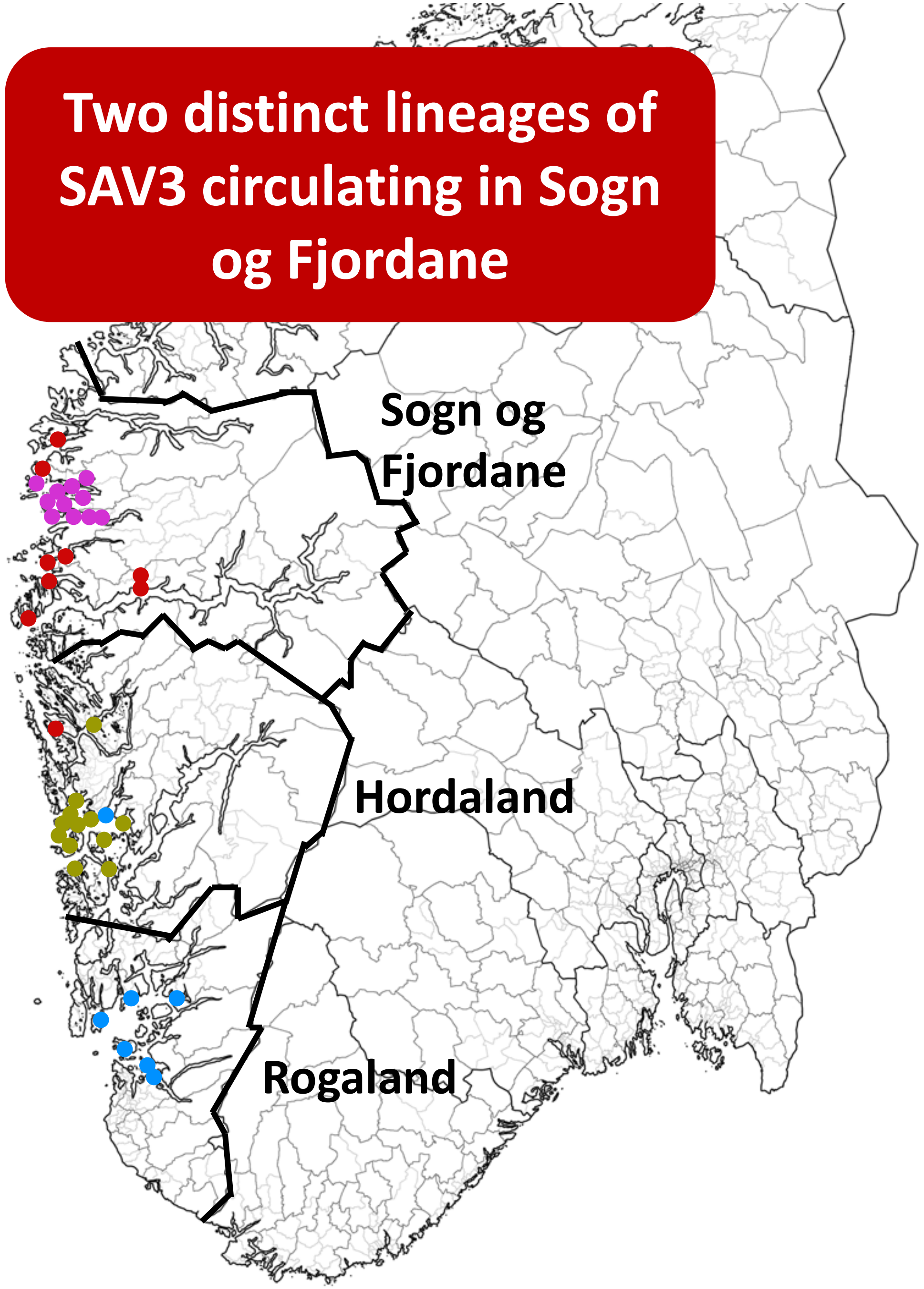
0.99 = posterior probability
0.99 = county probability

● = Hordaland reference sequences
● = Sogn og Fjordane reference sequences
● = Rogaland reference sequences



Seeding events from Hordaland into neighbouring counties

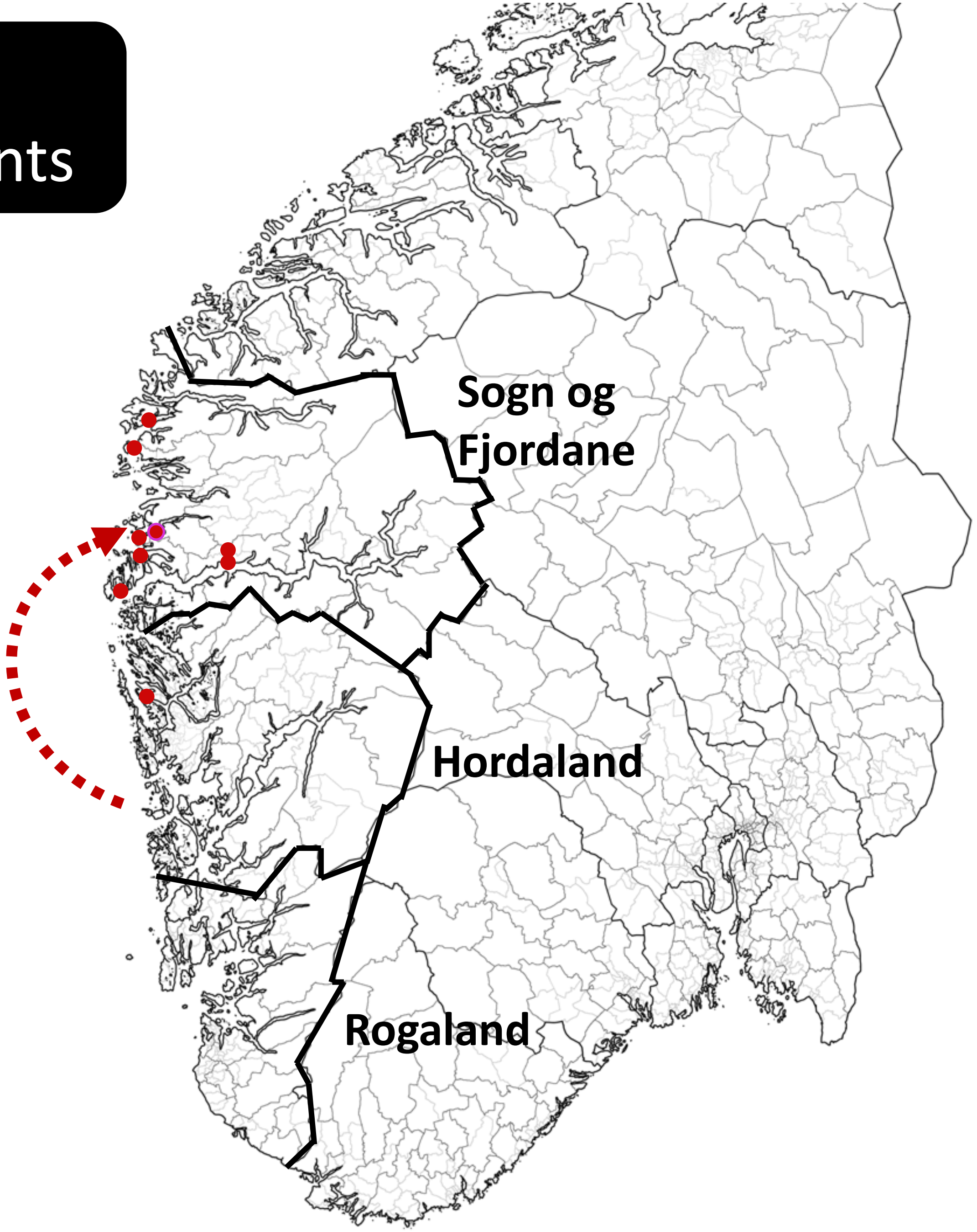
Two distinct lineages of SAV3 circulating in Sogn og Fjordane



SAV3 in Norway

Timing of seeding events

Movement of Sogn og Fjordane clade 1 between ~2020.0 and 2021.3

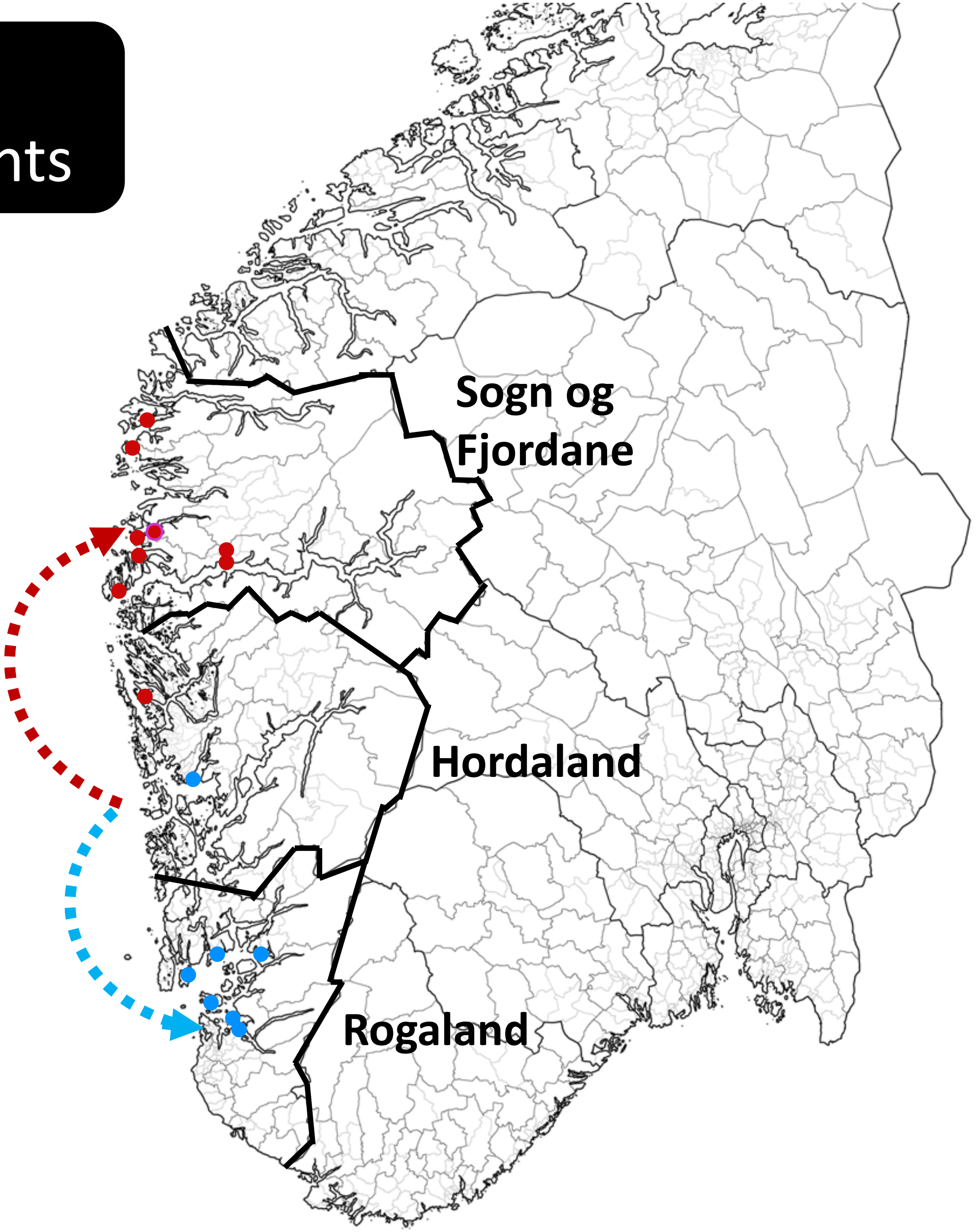


SAV3 in Norway

Timing of seeding events

Movement of Sogn og Fjordane clade 1 between ~2020.0 and 2021.3

Movement of Rogaland clade between ~2020.7 and 2021.4



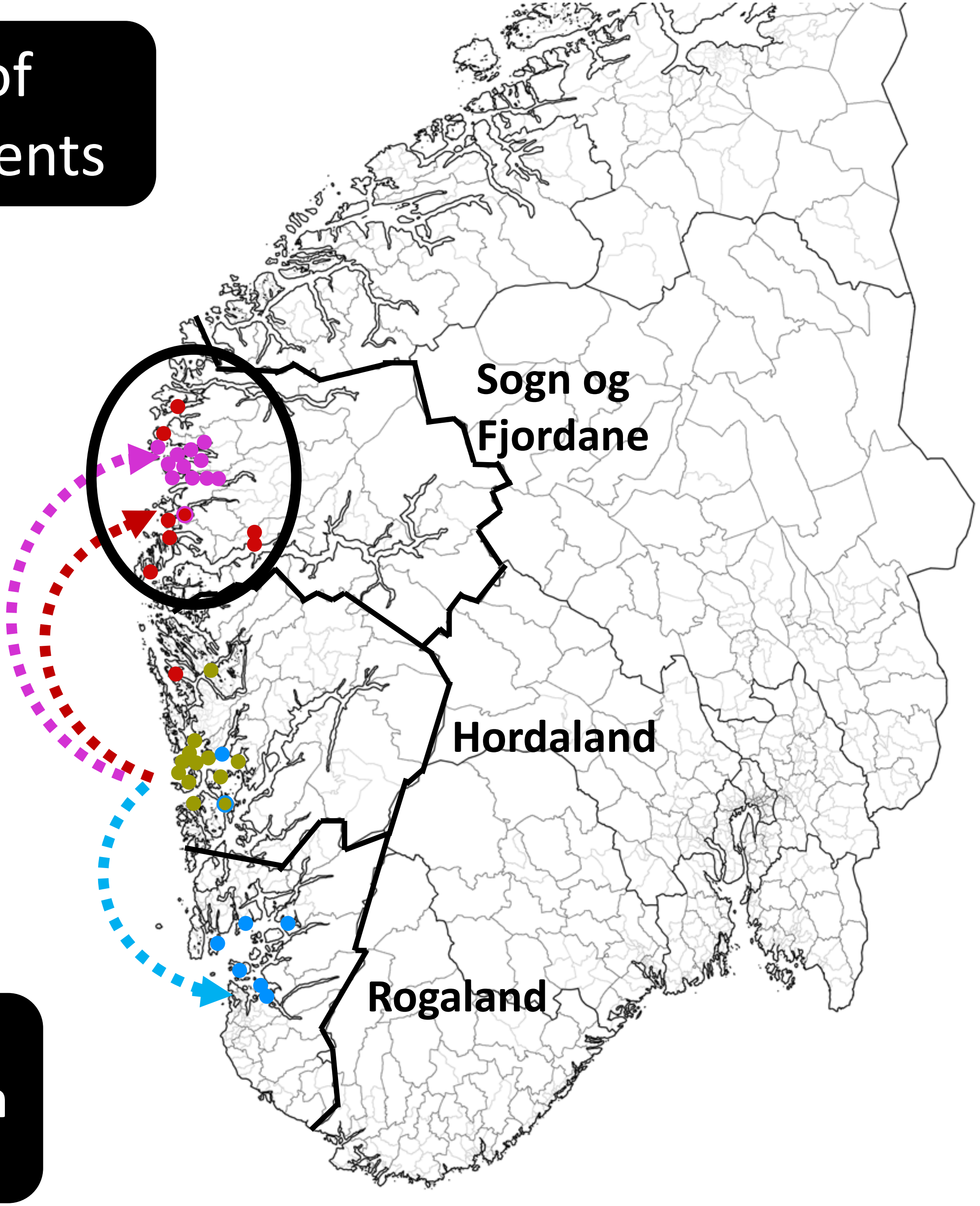
SAV3 in Norway

Timing of seeding events

Movement of Sogn og Fjordane clade 1 between ~2020.0 and 2021.3

Movement of Rogaland clade between ~2020.7 and 2021.4

Movement of Sogn og Fjordane clade 2 between ~2018.3 and 2022.3

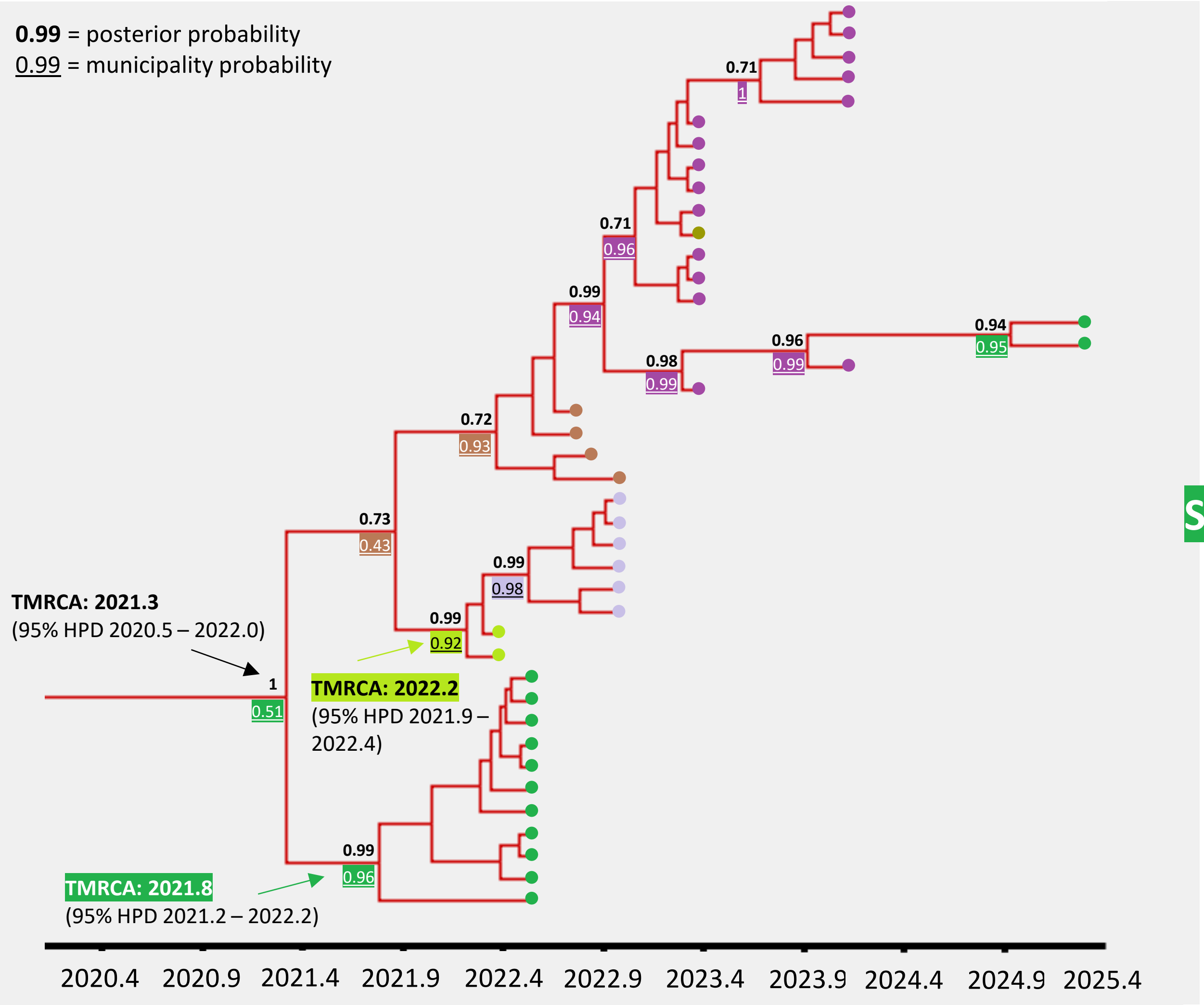


Can use genomic data to reconstruct viral transmission patterns on a finer scale

SAV3 in Norway

Strong support for presence in **Solund** by ~2021.8

Discrete trait analysis (municipality data) – Clade 1

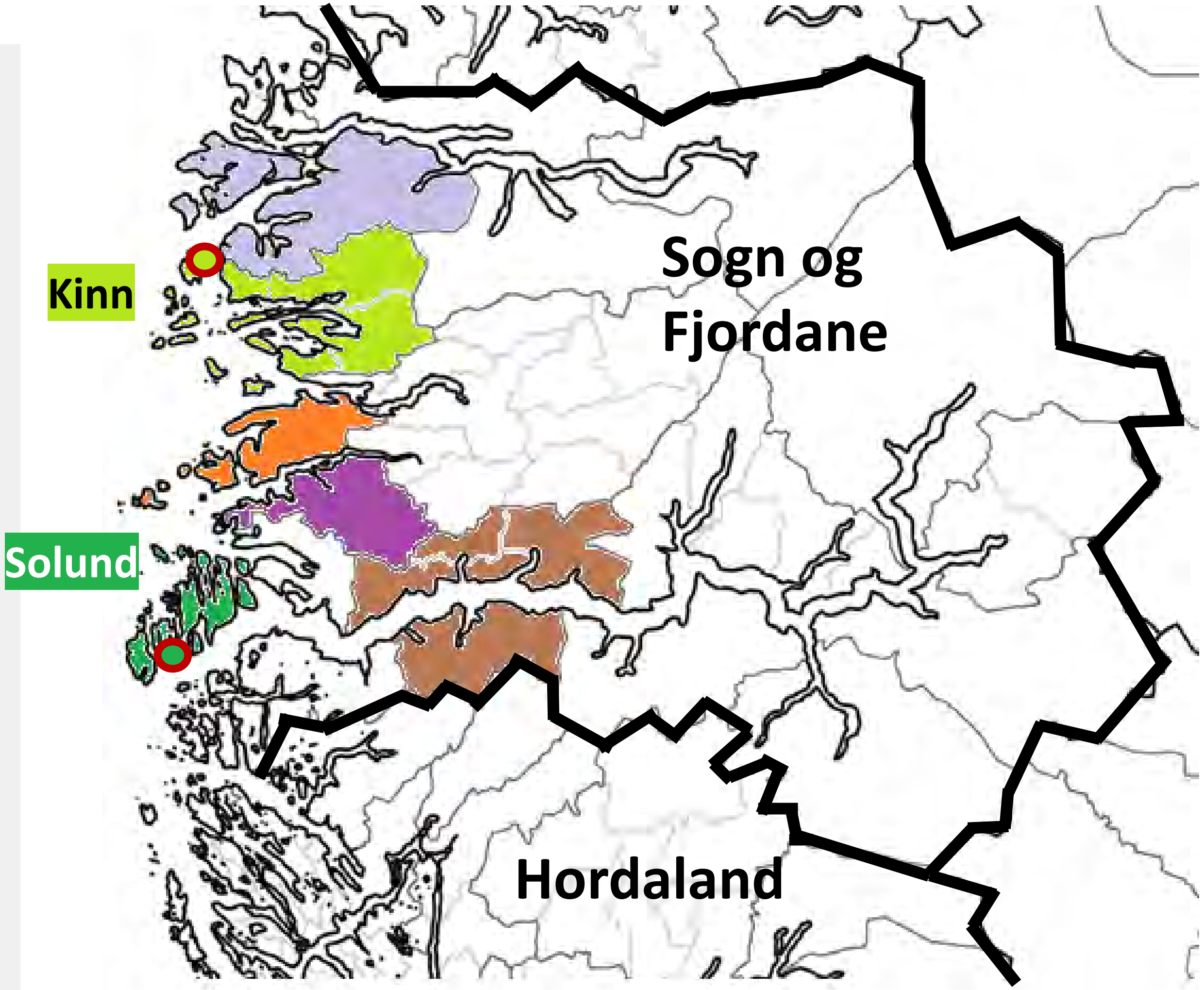


0.99 = posterior probability
0.99 = municipality probability

TMRCA: 2021.3
(95% HPD 2020.5 – 2022.0)

TMRCA: 2022.2
(95% HPD 2021.9 – 2022.4)

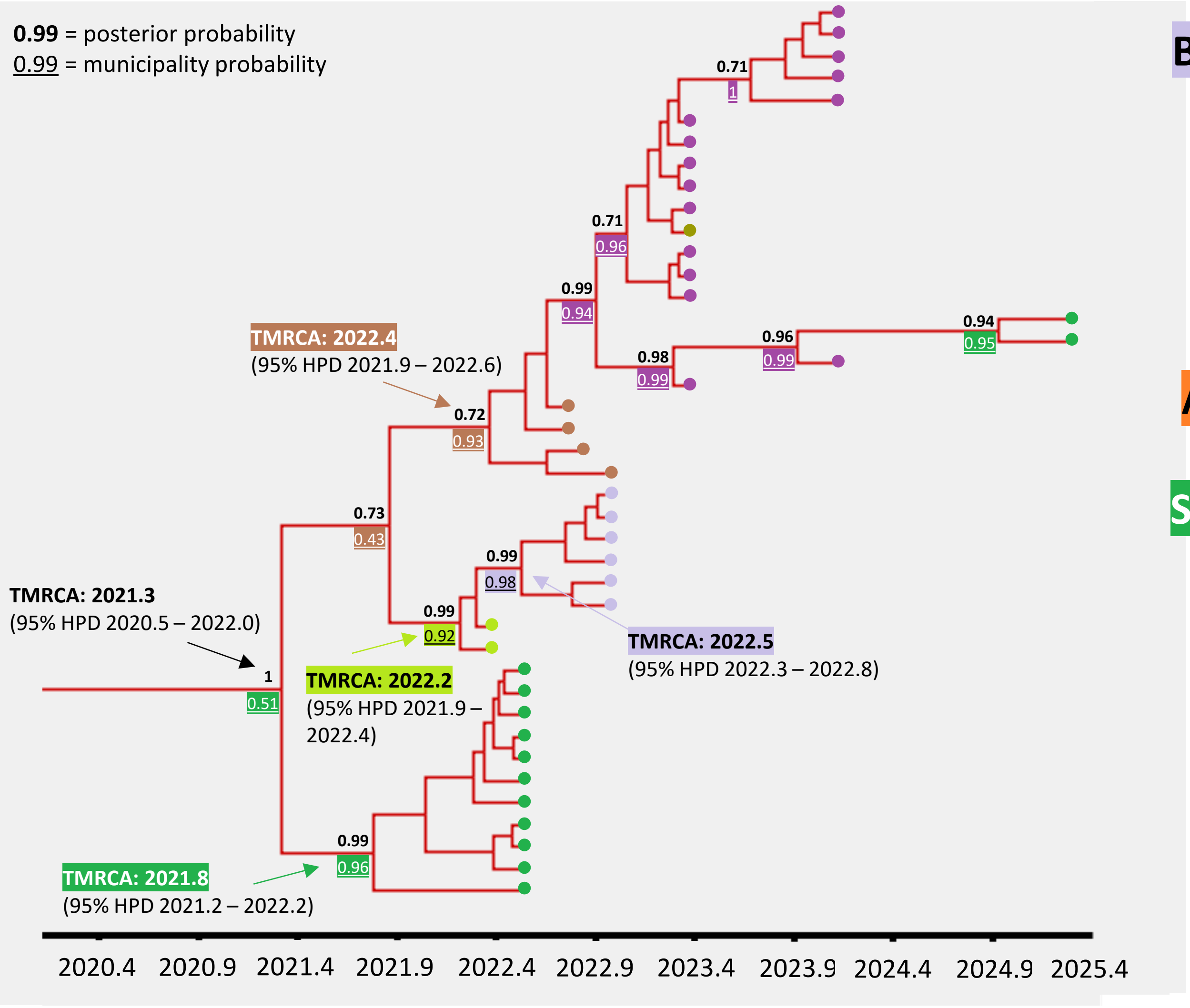
TMRCA: 2021.8
(95% HPD 2021.2 – 2022.2)

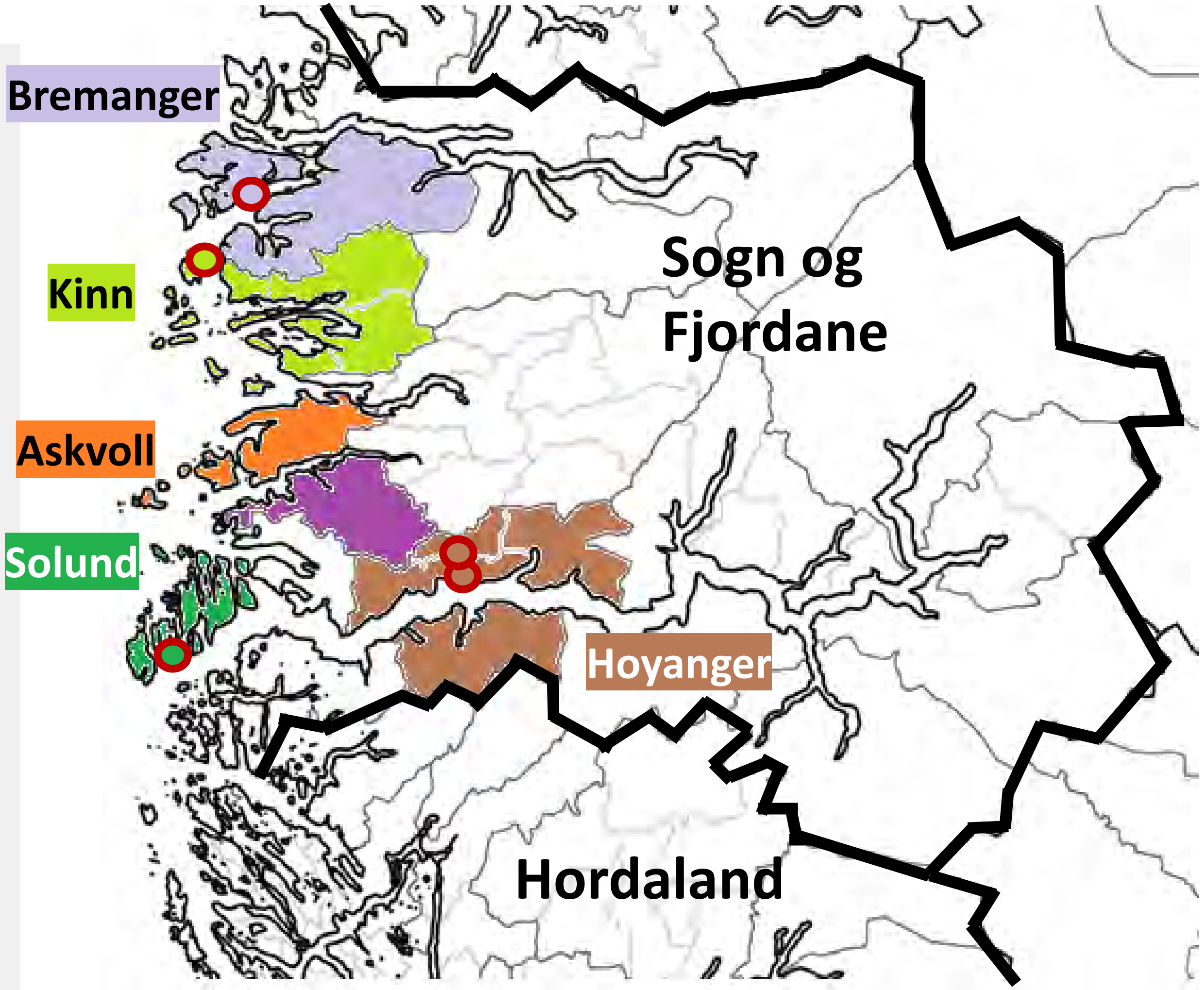


SAV3 in Norway

Clade detected in **Hoyanger** (~2022.4) and **Bremanger** (~2022.5)

Discrete trait analysis (municipality data) – Clade 1

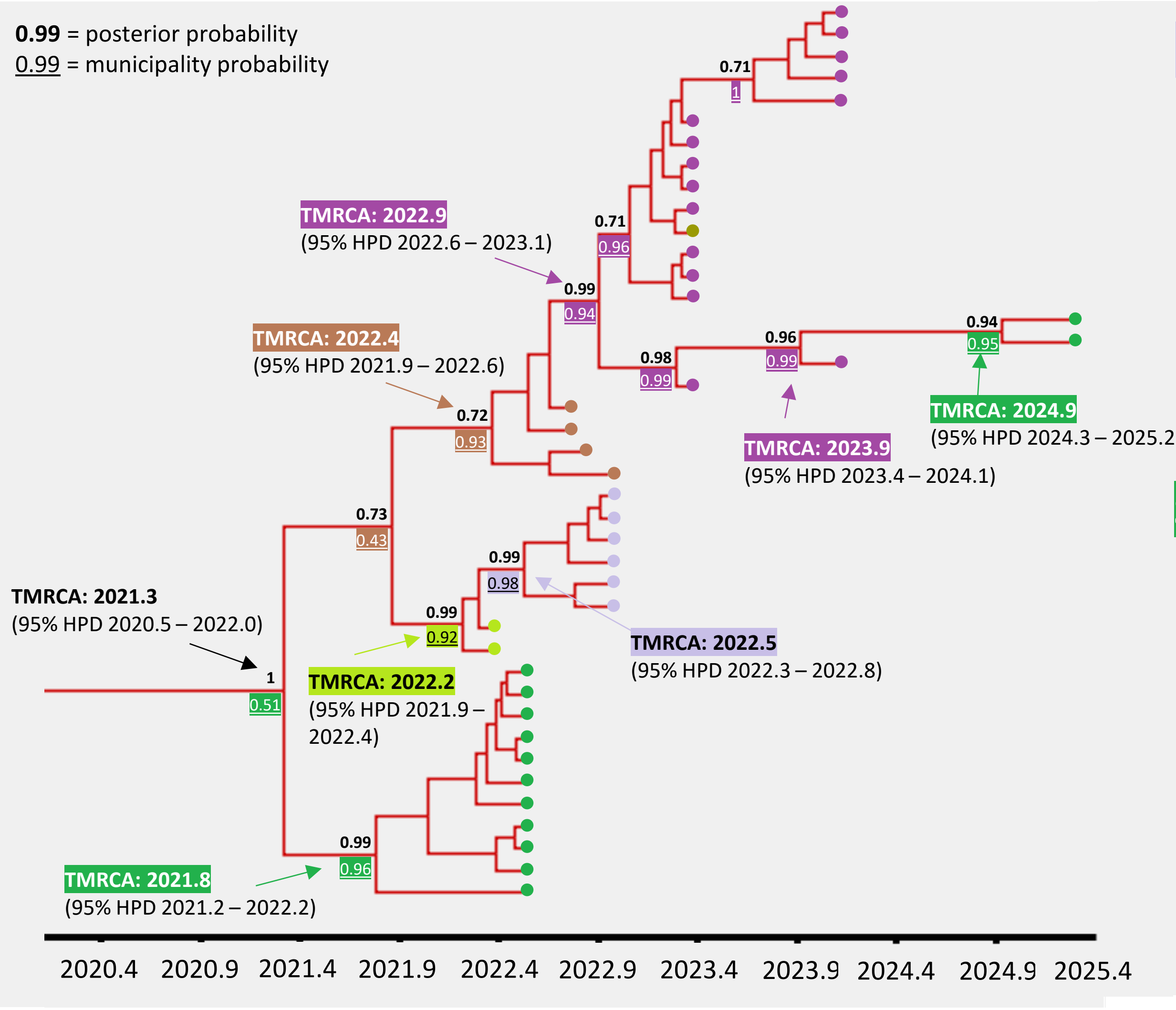




SAV3 in Norway

Clade detected in Fjaler (~2022.9) – still detected in municipality until at least 2024.2

Discrete trait analysis (municipality data) – Clade 1


0.99 = posterior probability

0.99 = municipality probability

TMRCA: 2022.9
(95% HPD 2022.6 – 2023.1)

TMRCA: 2022.4
(95% HPD 2021.9 – 2022.6)

TMRCA: 2023.9
(95% HPD 2023.4 – 2024.1)

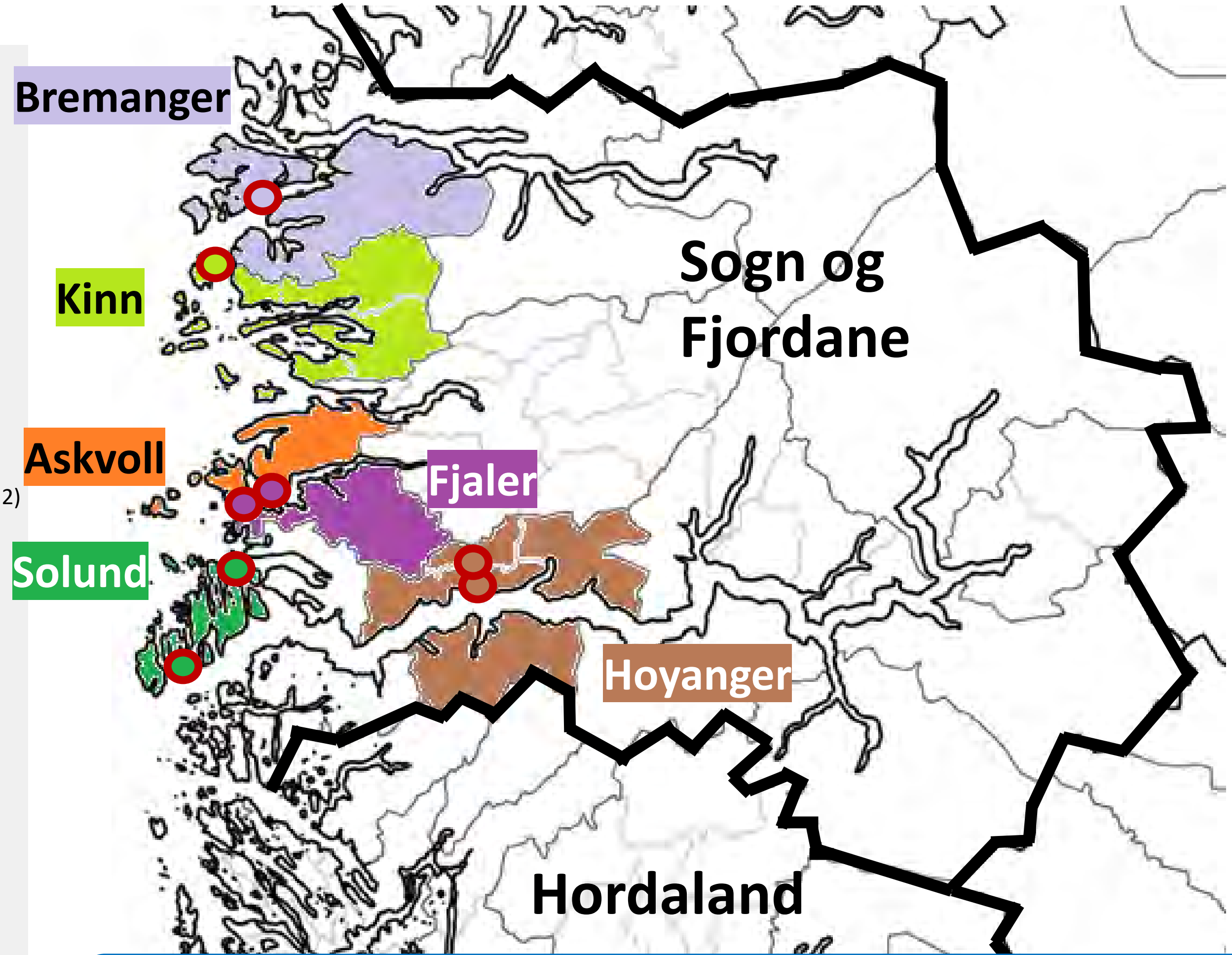
TMRCA: 2024.9
(95% HPD 2024.3 – 2025.2)

TMRCA: 2021.3
(95% HPD 2020.5 – 2022.0)

TMRCA: 2022.2
(95% HPD 2021.9 – 2022.4)

TMRCA: 2022.5
(95% HPD 2022.3 – 2022.8)

TMRCA: 2021.8
(95% HPD 2021.2 – 2022.2)

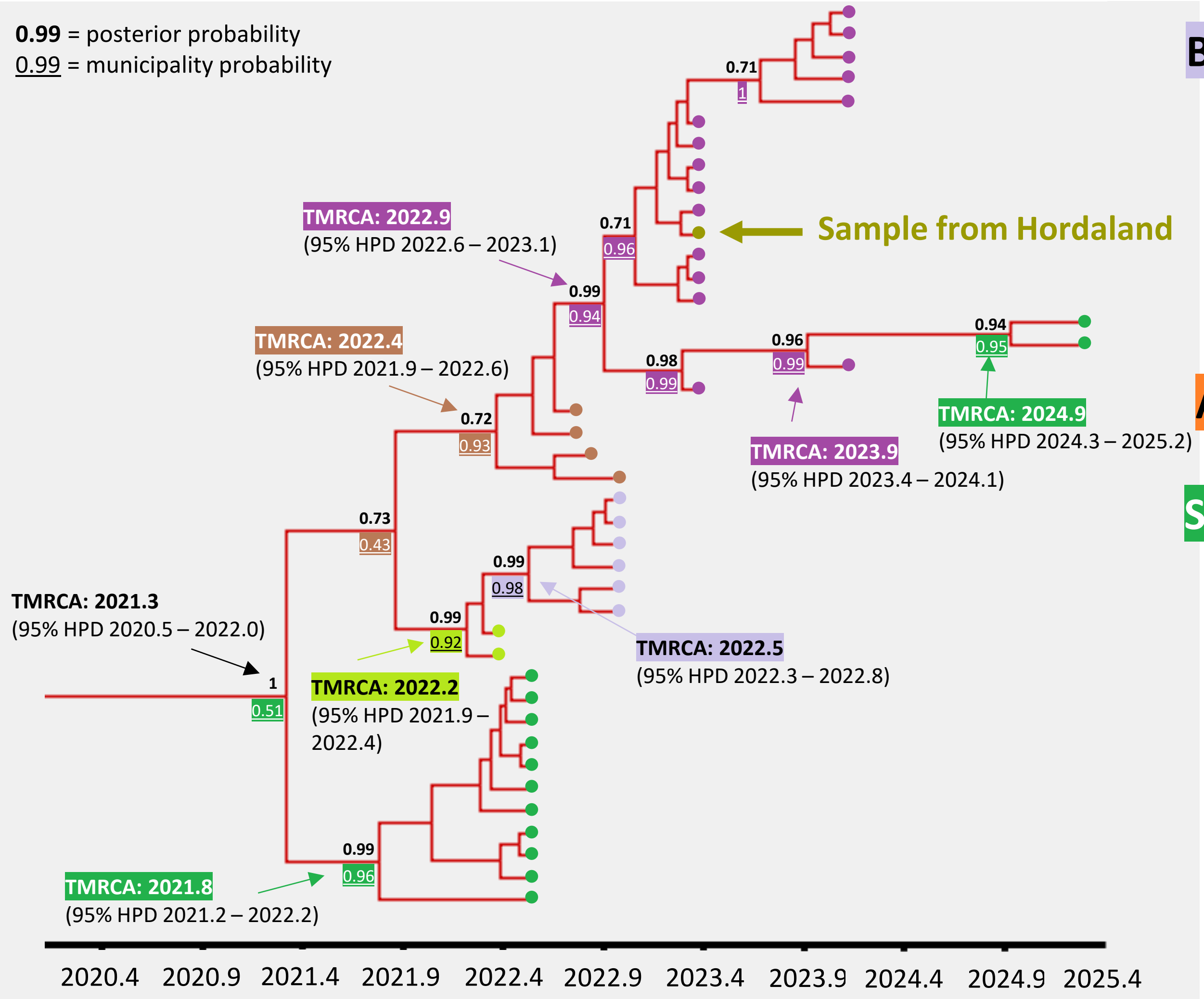


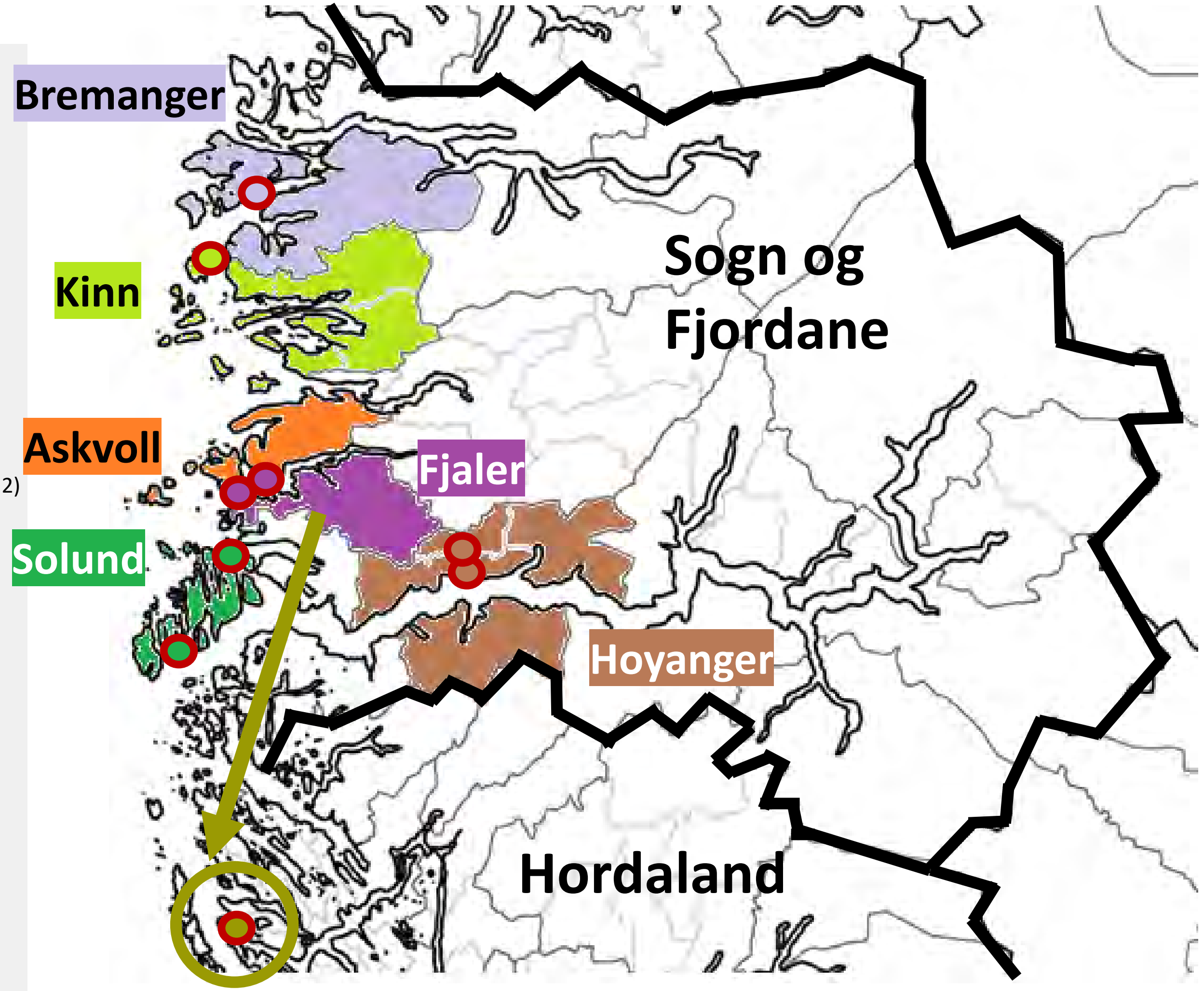
Lineage detected in Fjaler (~2023.9) later detected in neighbouring Solund in ~2024.9

SAV3 in Norway

Transmission from Sogn og Fjordane to Hordaland – outbreaks separated by a day

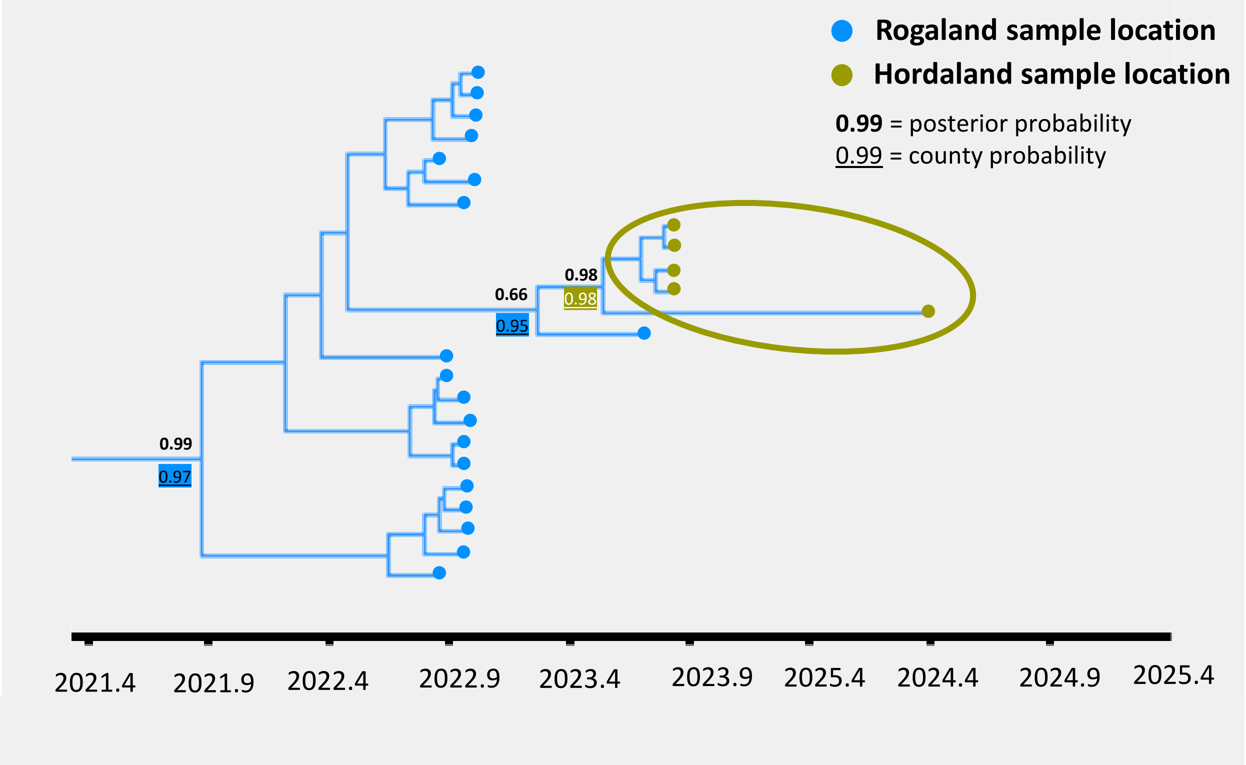
Discrete trait analysis (municipality data) – Clade 1

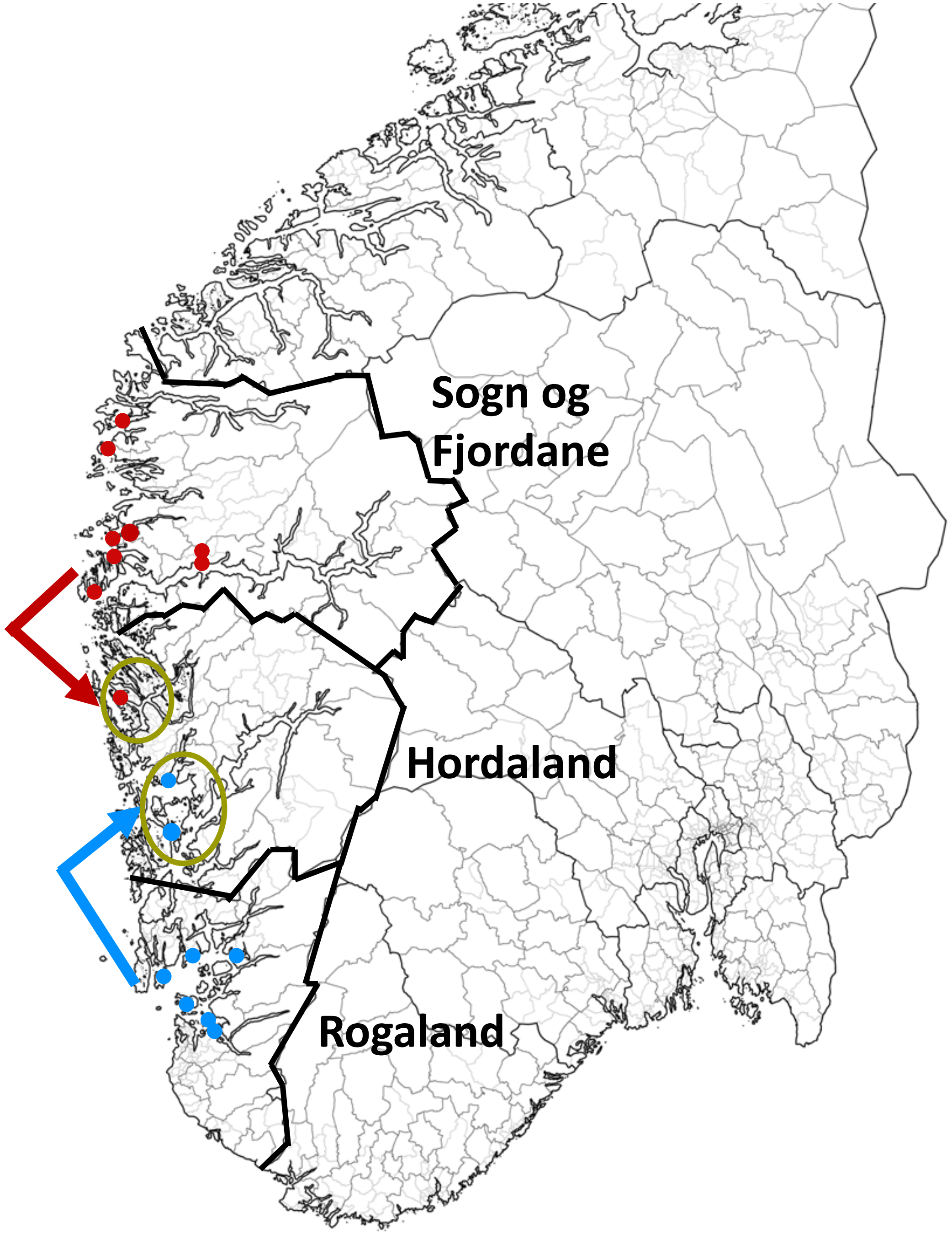




SAV3 in Norway

Discrete trait analysis (county data) – Rogaland clade

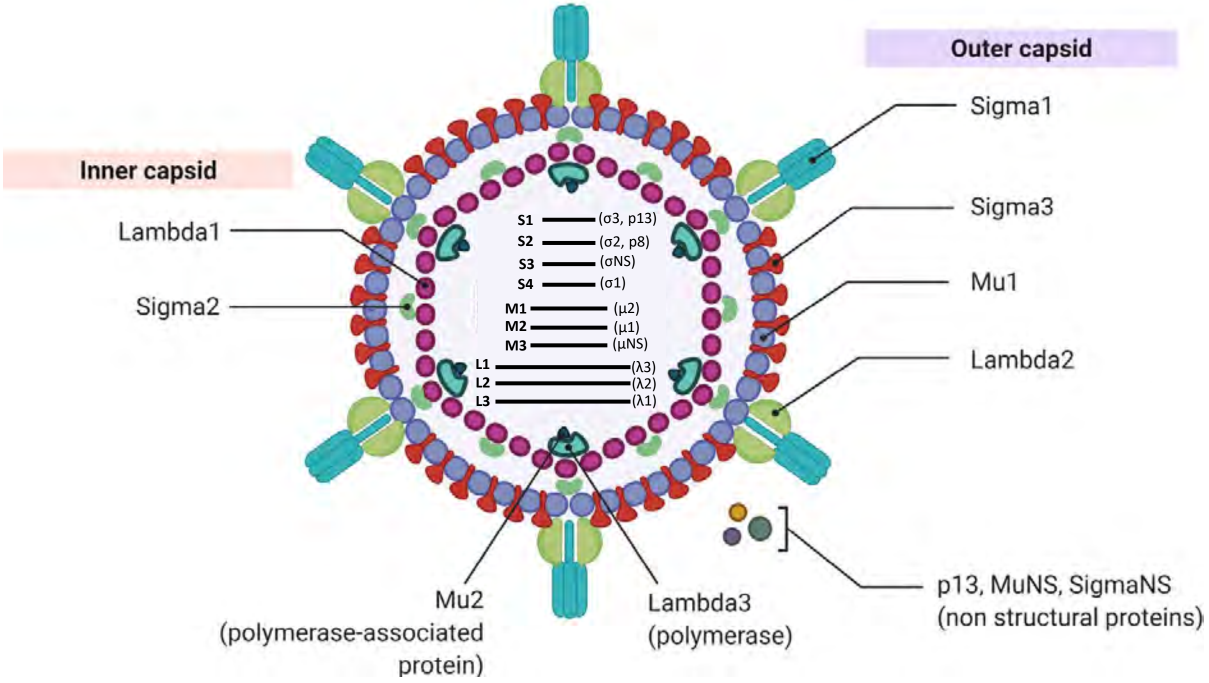




Seeding events detected from Sogn og Fjordane/Rogaland back into Hordaland

Piscine Orthoreovirus (PRV)

- Causative agent of Heart and Skeletal Muscle Inflammation (HSMI)
- Genome:
  - 10 segments
- Genotypes:
  - PRV1:
    - PRV1-Pacific
    - PRV1-Atlantic
  - PRV2 + PRV3



Adapted from: Vallejos-Vidal et al. 2022 (https://doi.org/10.3389/fimmu.2022.768621)

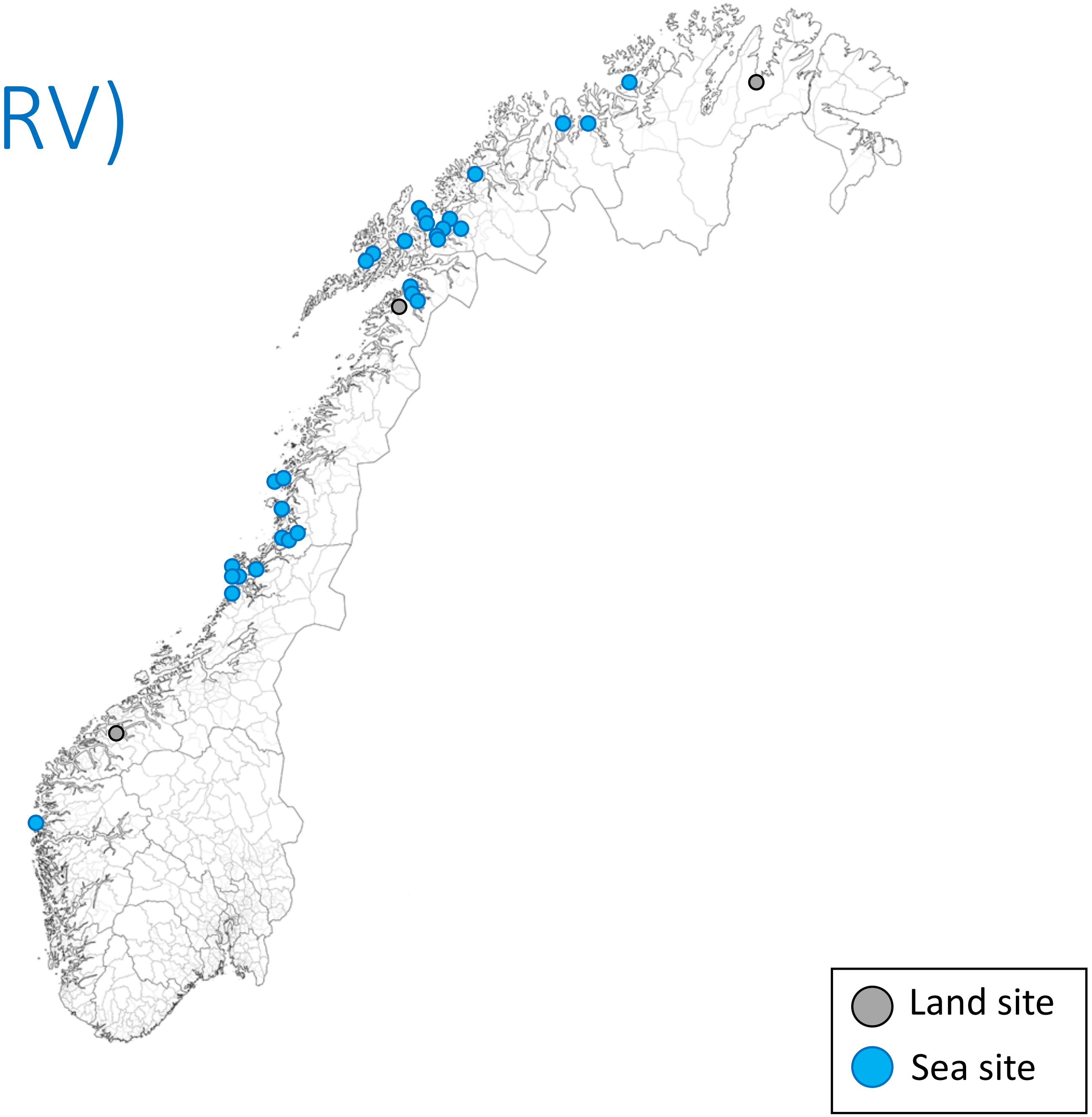
Producer locations (PRV)

No. of samples: 93
- Norway: 92
- Scotland: 1

No. of producers: 16
- No. of sites: 34
  - Land: 3
  - Sea: 31

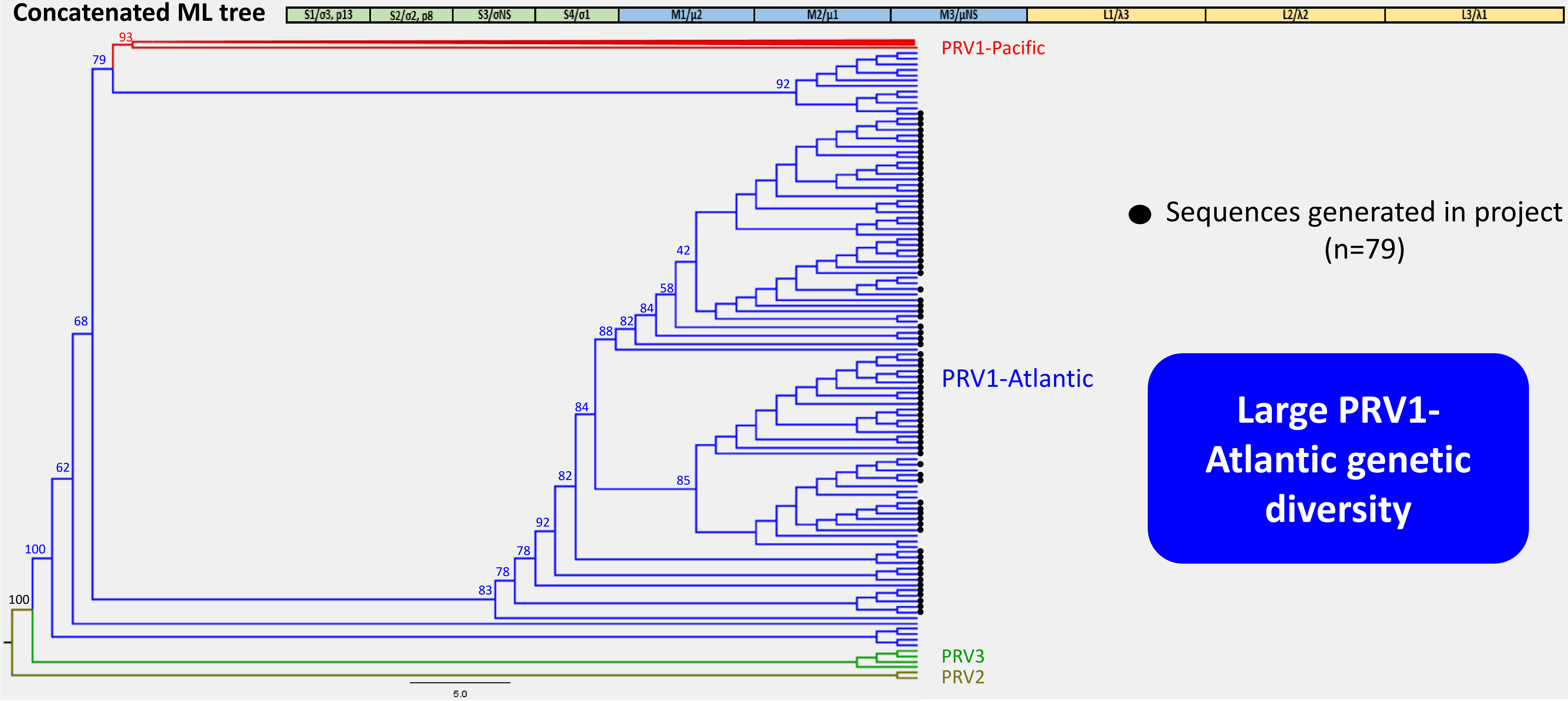
No. of outbreaks: 42

Date range: June 2023 – Dec 2023



PRV concatenated genome analysis

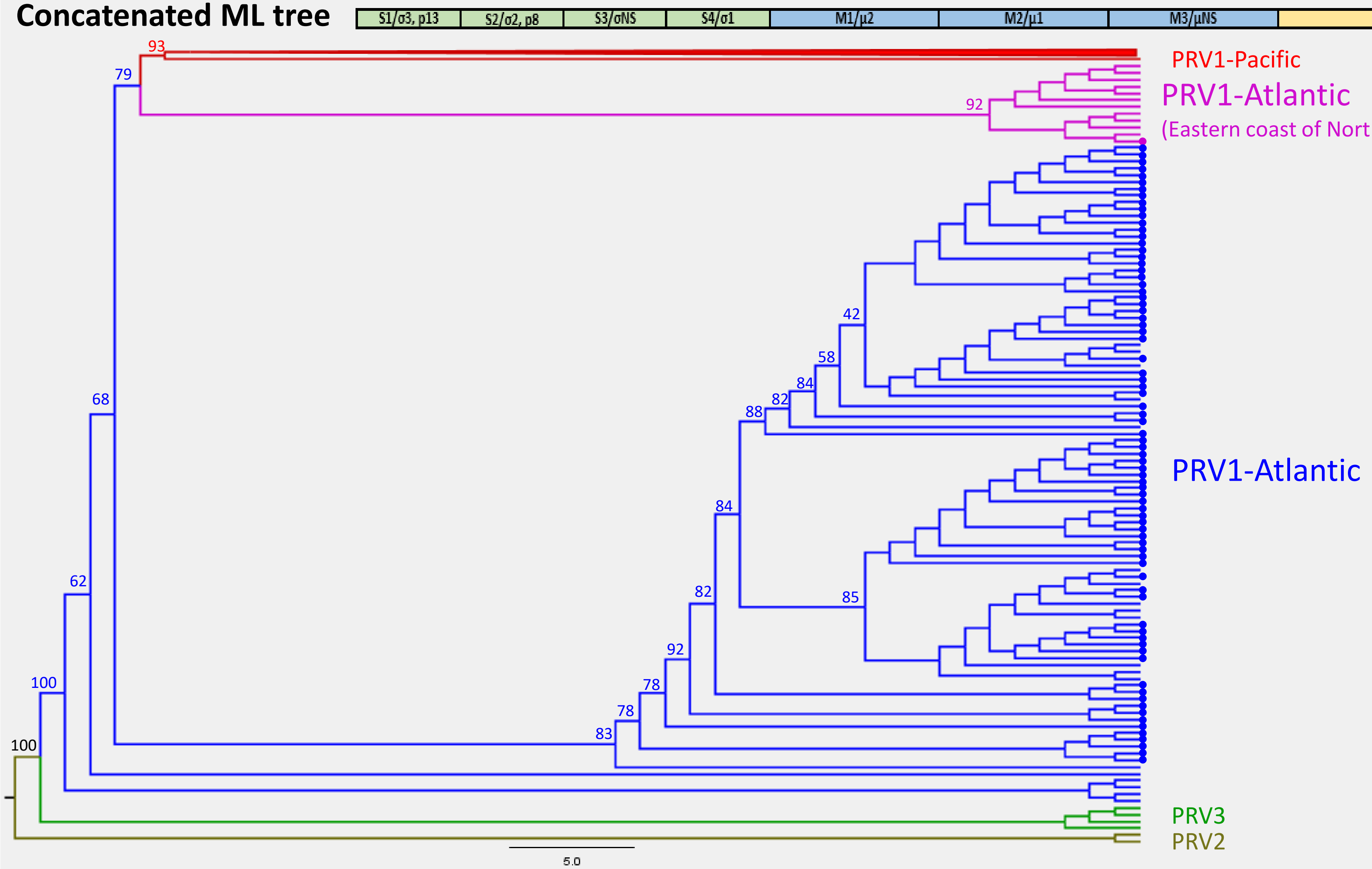
Concatenated ML tree



● Sequences generated in project (n=79)

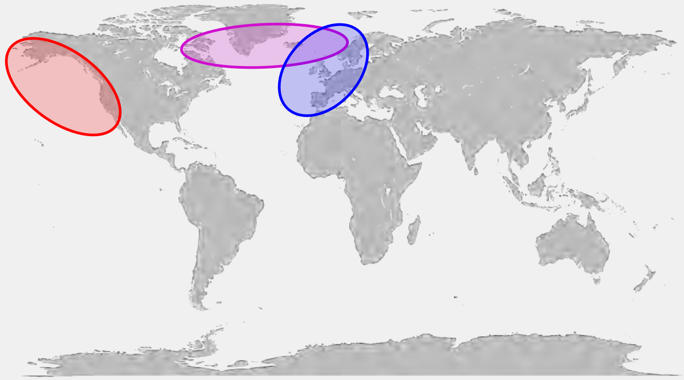
Large PRV1-Atlantic genetic diversity

PRV concatenated genome analysis



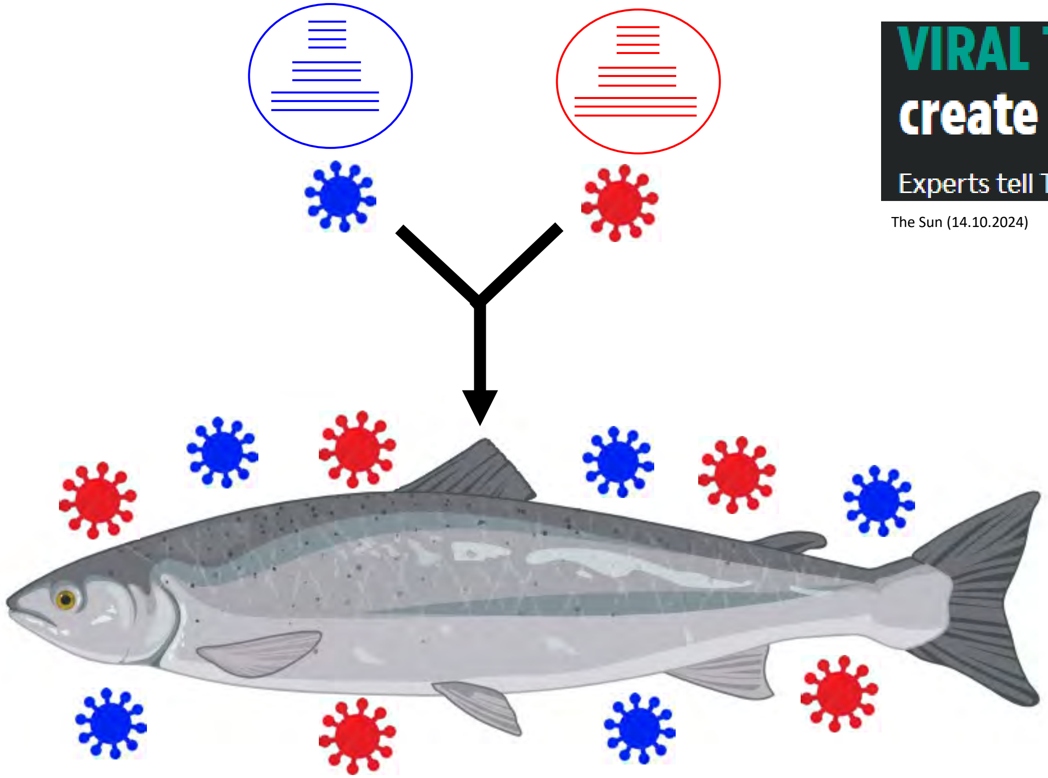
Concatenated ML tree

● Sequences generated in project (n=79)

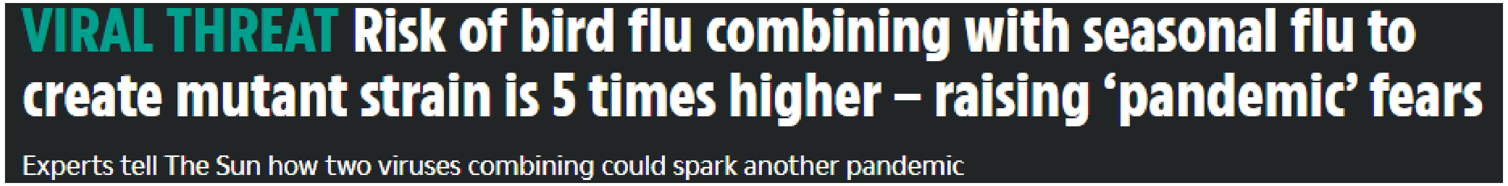


Reassortment

Coinfection with two variants



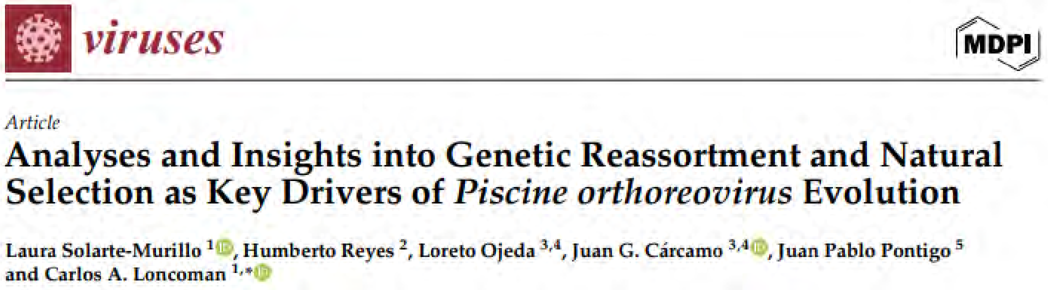
Monitoring reassortment combinations routine in human viruses e.g. influenza



VIRAL THREAT Risk of bird flu combining with seasonal flu to create mutant strain is 5 times higher – raising 'pandemic' fears

Experts tell The Sun how two viruses combining could spark another pandemic

The Sun (14.10.2024)



viruses

MDPI

Article

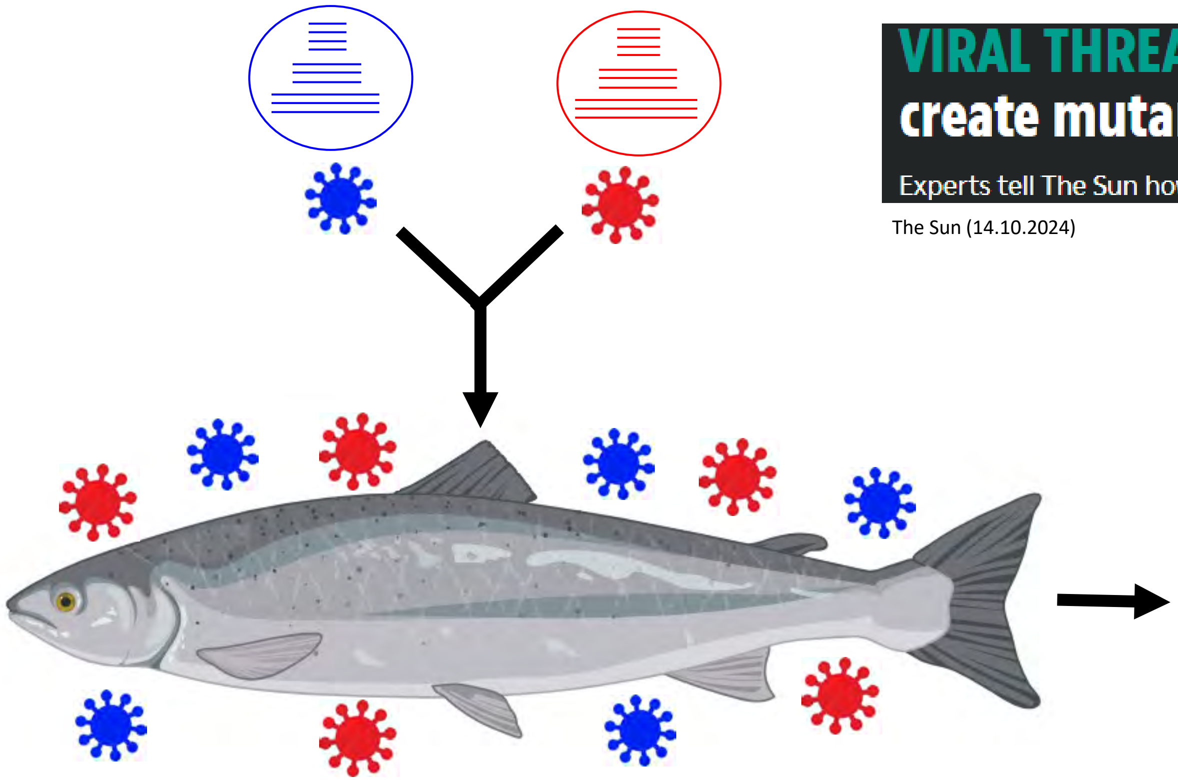
Analyses and Insights into Genetic Reassortment and Natural Selection as Key Drivers of *Piscine orthoreovirus* Evolution

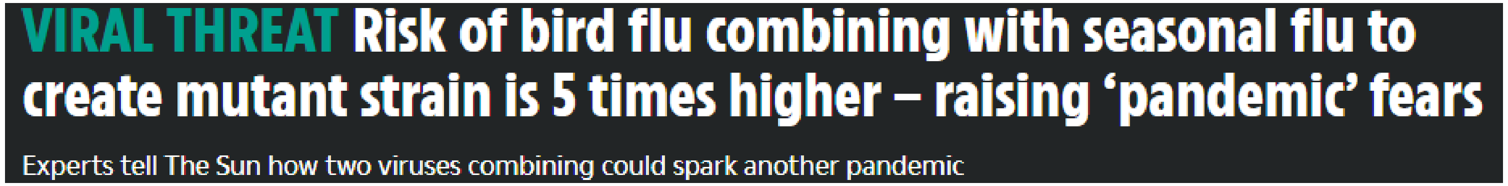
Laura Solarte-Murillo [1], Humberto Reyes [2], Loreto Ojeda [3,4], Juan G. Cárcamo [3,4], Juan Pablo Pontigo [5] and Carlos A. Loncoman [1,*]

Reassortment

Monitoring reassortment combinations routine in human viruses e.g. influenza

Coinfection with two variants



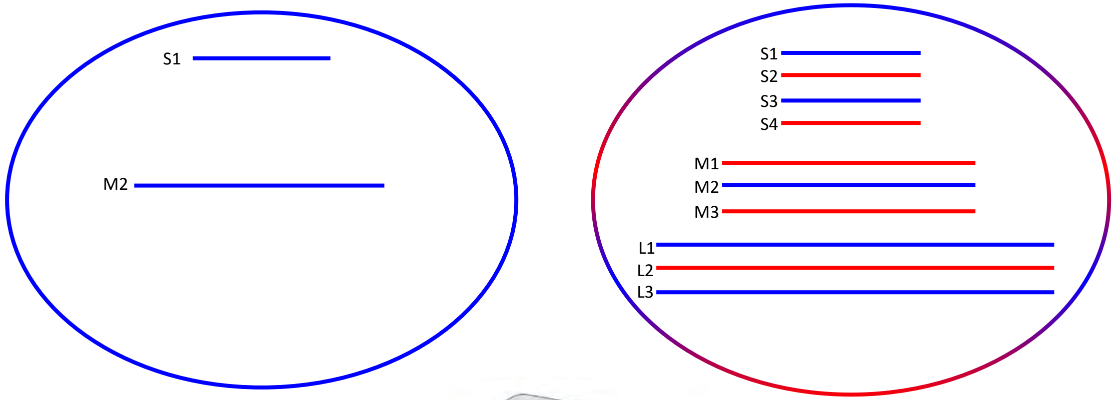


VIRAL THREAT Risk of bird flu combining with seasonal flu to create mutant strain is 5 times higher – raising 'pandemic' fears

Experts tell The Sun how two viruses combining could spark another pandemic

The Sun (14.10.2024)

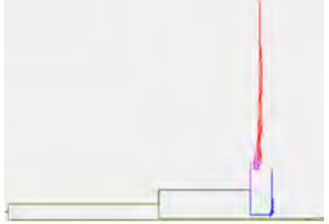
Standard sequencing for diagnostics **Whole genome sequencing**

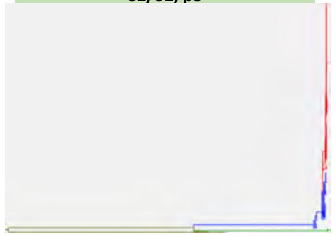


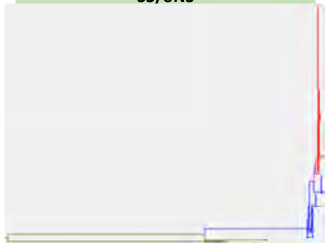
Potential reassortant

Whole genome sequencing provides a greater genetic and reassortment characterisation than standard genotyping sequencing

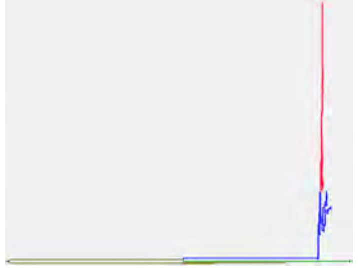
Reassortment


S1/σ3, p13


S2/σ2, p8

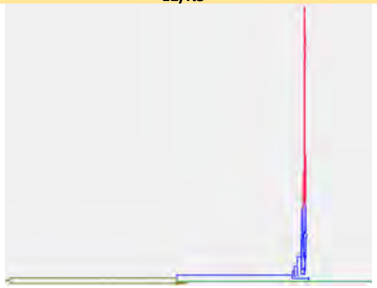

S3/σNS

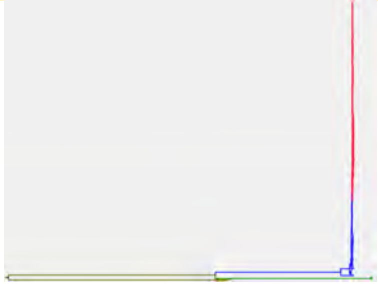

S4/σ1

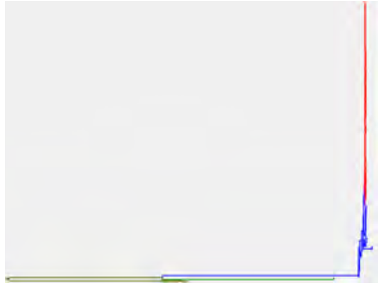

M1/μ2


M2/μ1


M3/μNS


L1/λ3

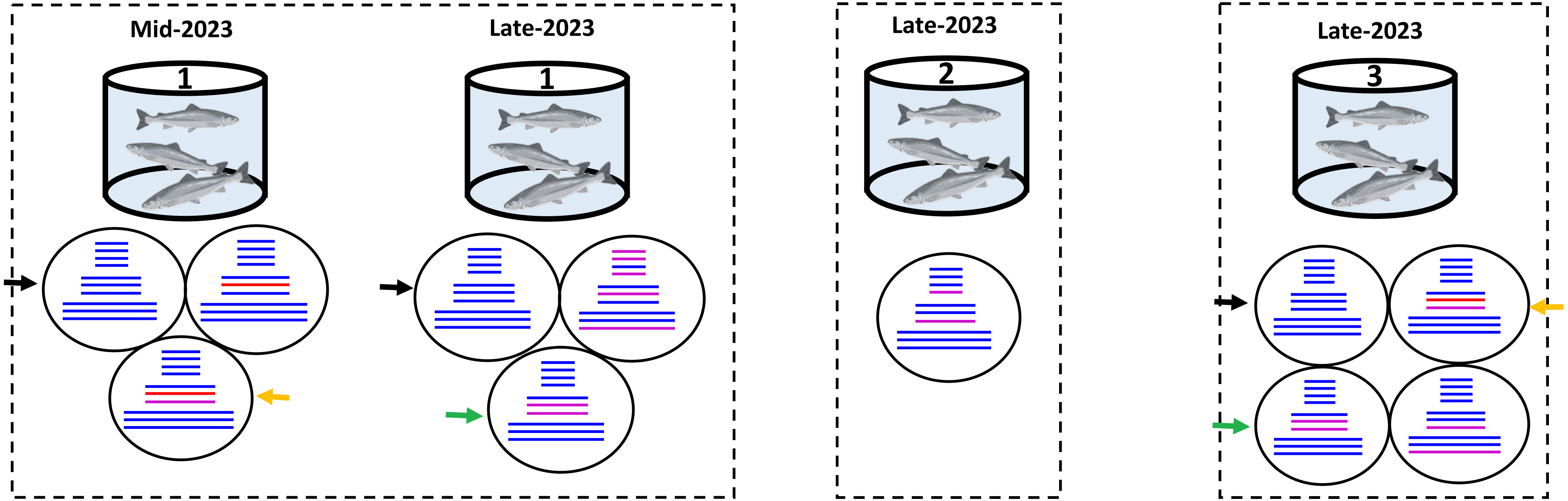

L2/λ2


L3/λ1

Comparing phylogenetic relationship of sample segments reveals reassortment events

Outbreaks contain diverse PRV reassortants

Four outbreaks across 3 producers (within 15km of each other)

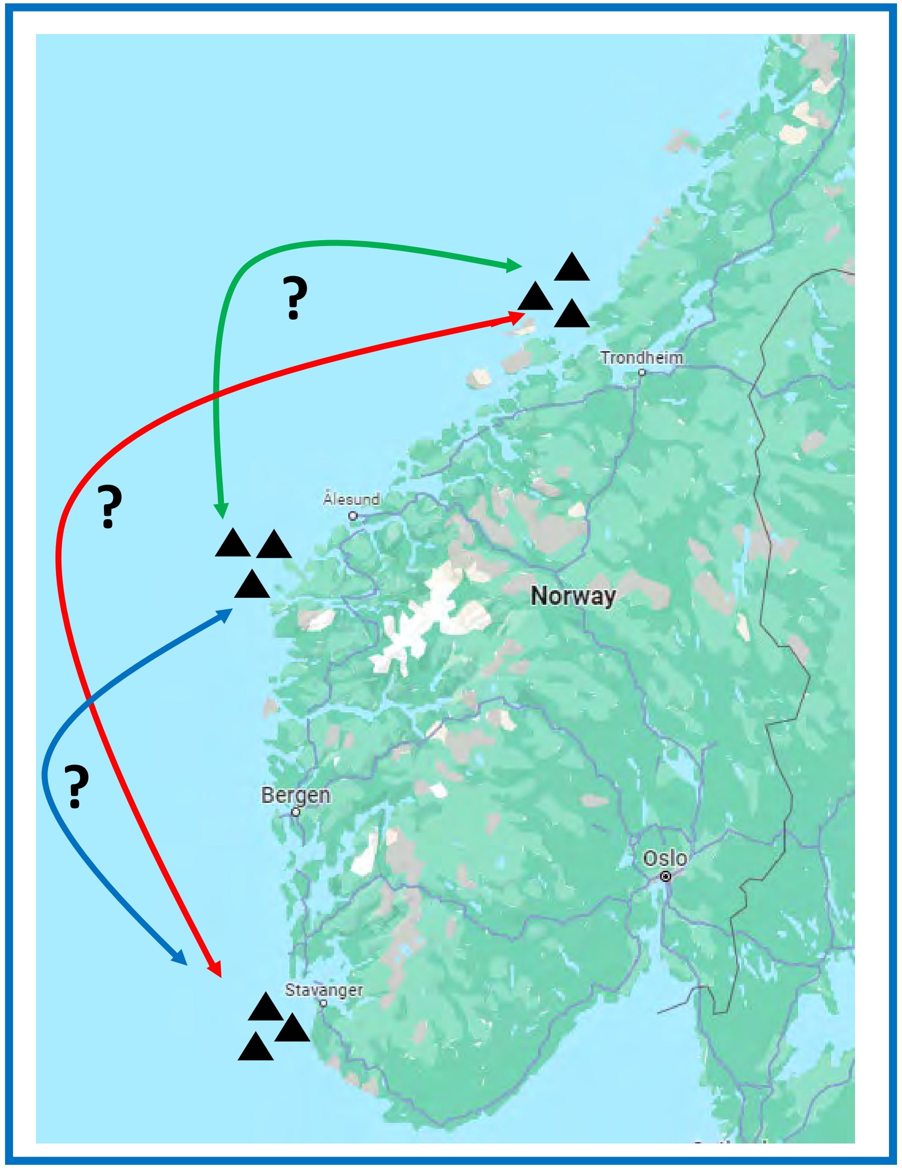


Large number of reassorted PRV detected within small area and short time frame

Reassortment can help understanding of PRV virulence and transmission dynamics

Conclusions

- Whole genome sequencing can be used for reconstructing viral transmission dynamics
  - Aid infection control decision making
- Reassortment monitoring key in understanding PRV diversity and evolution



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PHARMAQ
part of zoetis


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Marte Follesø Sønnervik


Marius Karlsen


Svein Alexandersen


Elise Hjelle

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UKRI
Biotechnology and Biological Sciences Research Council

SAIC
Sustainable Aquaculture Innovation Centre