Genome sequencing for epidemiological studies of SAV2 and SAV3 in Norway

Dan Macqueen [1] *,
Svein Alexandersen [2] ,
Marius Karlsen [2], *et al.*

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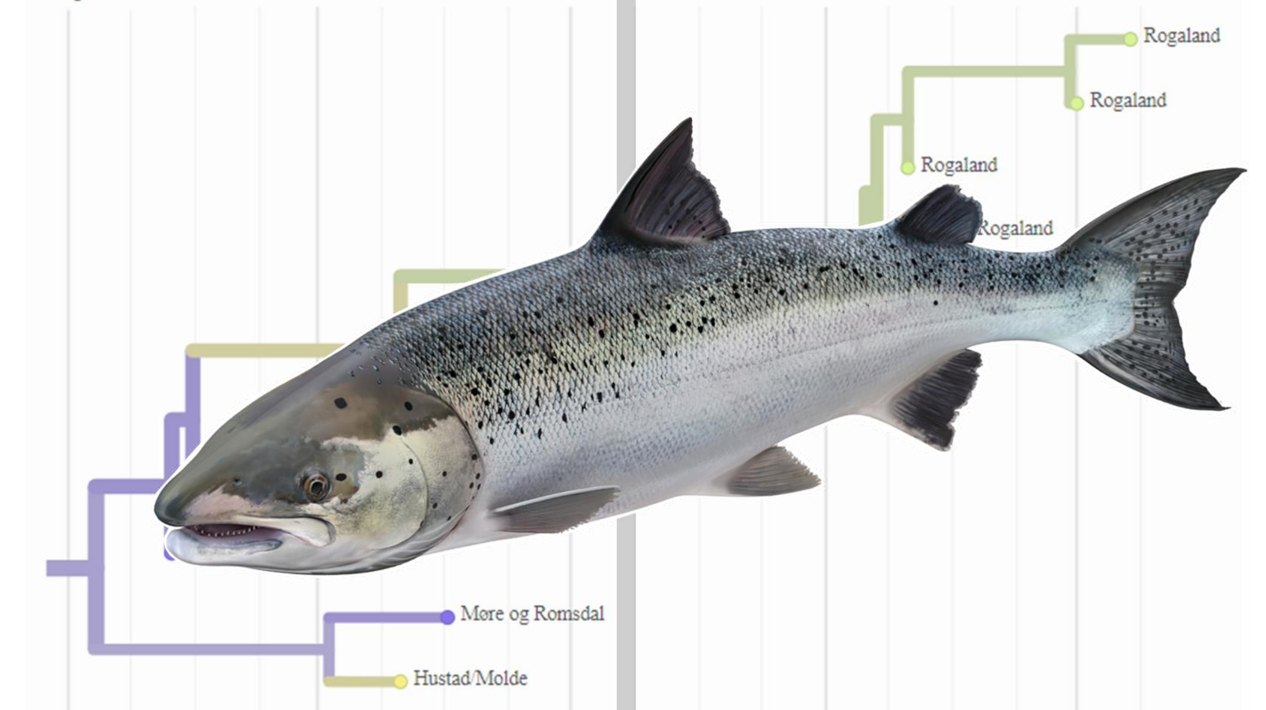


1
ROSLIN
THE UNIVERSITY of EDINBURGH
The Royal (Dick) School
of Veterinary Studies

2
PHARMAQ
part of zoetis

TRINATION

Disease control strategies – including biosecurity and vaccination

23rd Nov 2022

Previous work on SAV genomics

BBSRC PhD – 2016-2020 (Dr Mike Gallagher)

Developed approaches for genome sequencing of SAV in salmonids – previous presentations at PD TriNation

- With Marine Scotland, Marine Institute and Pharmaq
- First collaboration with Pharmaq on SAV3

Mike Gallagher

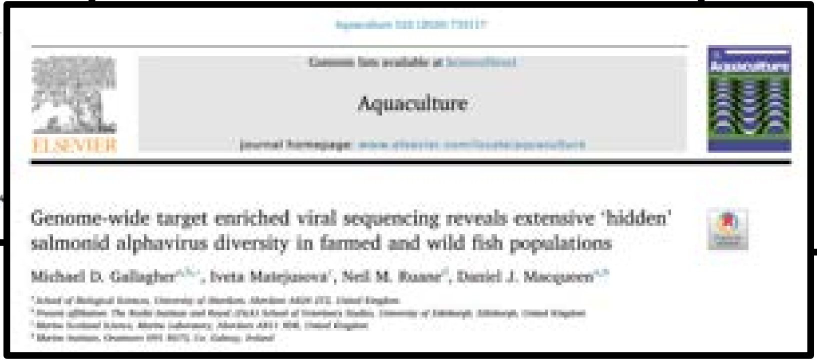
SCIENTIFIC REPORTS

OPEN

Nanopore sequencing for rapid diagnostics of salmonid RNA viruses

Michael D. Gallagher[1,5], Iveta Matejusova[2], Lien Nguyen[1], Neil M. Ruane[3], Knut Falk
Daniel J. Macqueen[1,5]

Gallagher et al. 2018. Sci. Rep. 8: 16307



Contents lists available at ScienceDirect

Aquaculture

Genome-wide target enriched viral sequencing reveals extensive 'hidden' salmonid alphavirus diversity in farmed and wild fish populations

Michael D. Gallagher, Iveta Matejusova, Neil M. Ruane, Daniel J. Macqueen

Gallagher et al. 2020. Aquaculture. 522: 735117

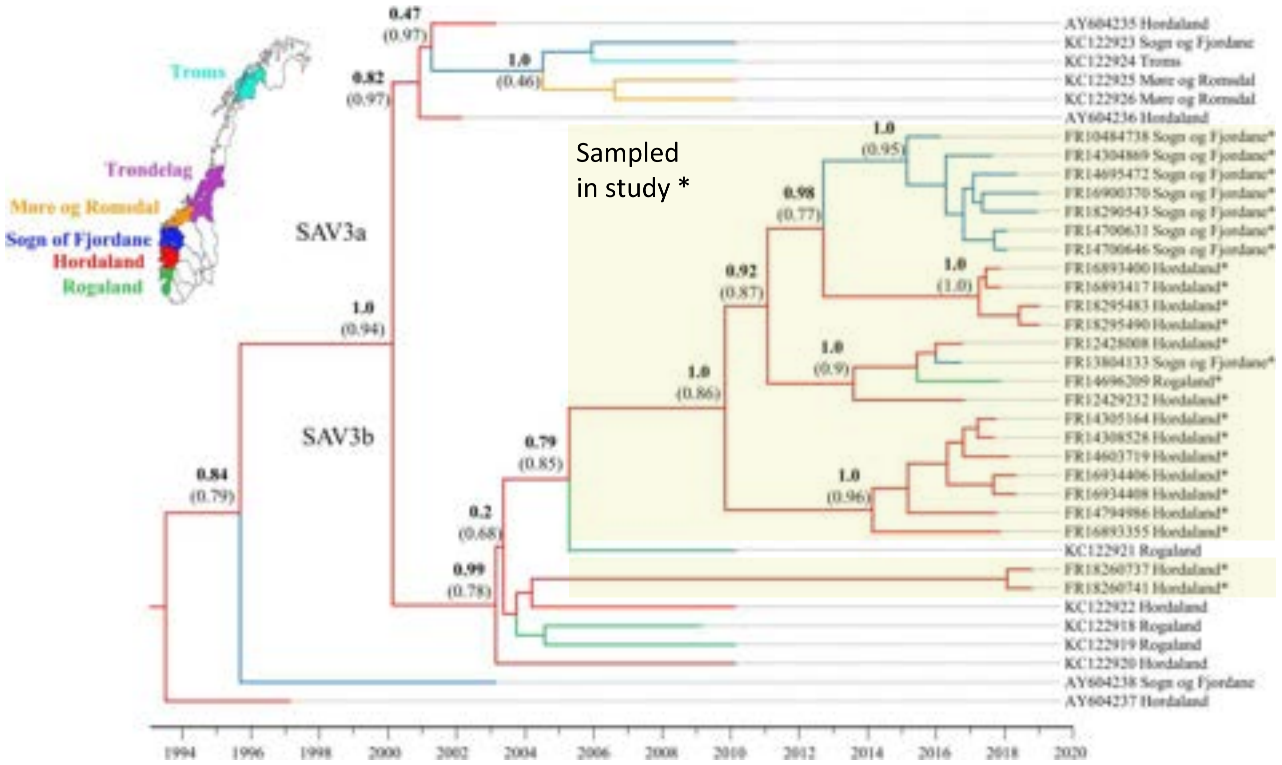
frontiers in Microbiology

Genome Sequencing of SAV3 Reveals Repeated Seeding Events of Viral Strains in Norwegian Aquaculture

Michael D. Gallagher, Marius Karlsen, Elin Petterson, Øyvind Haugland, Iveta Matejusova and Daniel J. Macqueen

Gallagher et al. 2020. Front. Microbiol. 11: 740.

SAV3 genome sequencing



Sampled
in study *

frontiers
in Microbiology

ORIGINAL RESEARCH
published: 24 April 2020
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Genome Sequencing of SAV3 Reveals Repeated Seeding Events of Viral Strains in Norwegian Aquaculture

Michael D. Gallagher[1,2*], Marius Karlsen[3], Elin Petterson[3], Øyvind Haugland[3], Iveta Matejusova[4] and Daniel J. Macqueen[2]

n=24 SAV3 genomes sequenced from field sites sampled 2016 - 2019 from 4 regions

Hordaland inferred as common source for SAV3 in recent circulation, in addition to lineages that may have been eradicated by control measures

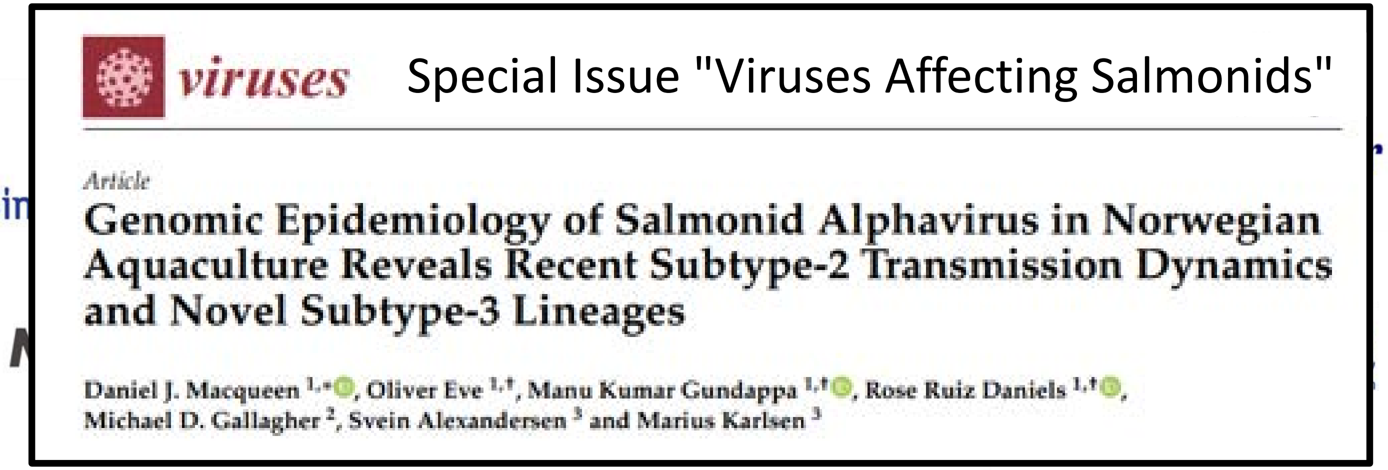
- Highlighted promise of genomic sequencing to infer SAV transmission dynamics, but lacked resolution

Upscaling SAV2 sequencing

Scientific objective: Reconstruct genetic diversity and transmission of SAV2 in Norwegian Atlantic salmon aquaculture using genome sequencing

Purpose: demonstrate strategy to support disease control

- Initiated by Sinkaberg Hansen and Pharmaq (Svein Alexandersen)
- Nine producers provided samples – *industry collaboration was key to success*



viruses Special Issue "Viruses Affecting Salmonids"

Article

Genomic Epidemiology of Salmonid Alphavirus in Norwegian Aquaculture Reveals Recent Subtype-2 Transmission Dynamics and Novel Subtype-3 Lineages

Daniel J. Macqueen [1,*], Oliver Eve [1,†], Manu Kumar Gundappa [1,†], Rose Ruiz Daniels [1,†], Michael D. Gallagher [2], Svein Alexandersen [3] and Marius Karlsen [3]

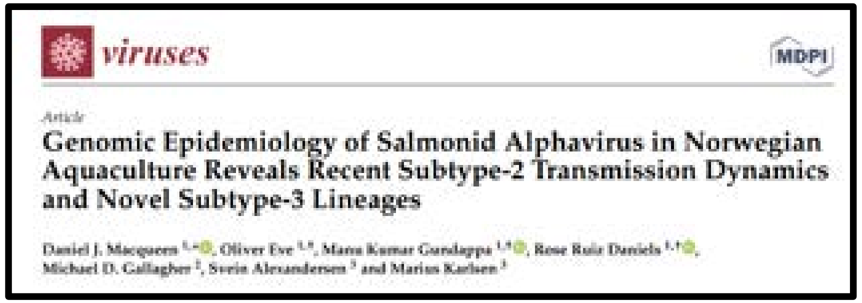
Overview of study

PHARMAQ part of zoetis

ROSLIN

~100 samples (heart) from 27 sampling sites across 16 production areas

- Metadata captured - sampling location & date, viral load, production region, etc.



viruses

MDPI

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Grieg Seafood

Alsaker Fjordbruk

Måsøval

SalmoNor

MOWI

OSLAND HAVBRUK

EMILSEN FISK AS

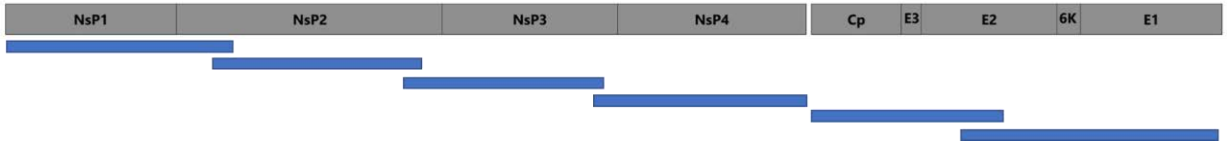
Midt-Norsk Havbruk AS

SALMAR Passion for Salmon

Producer identity anonymized:
inferences limited to farming region

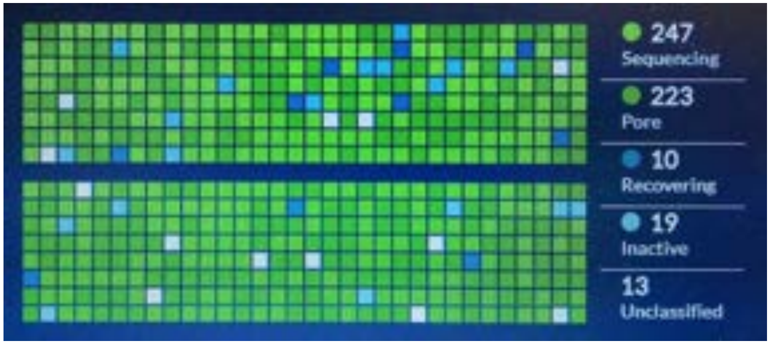
Sequencing of SAV2 genomes

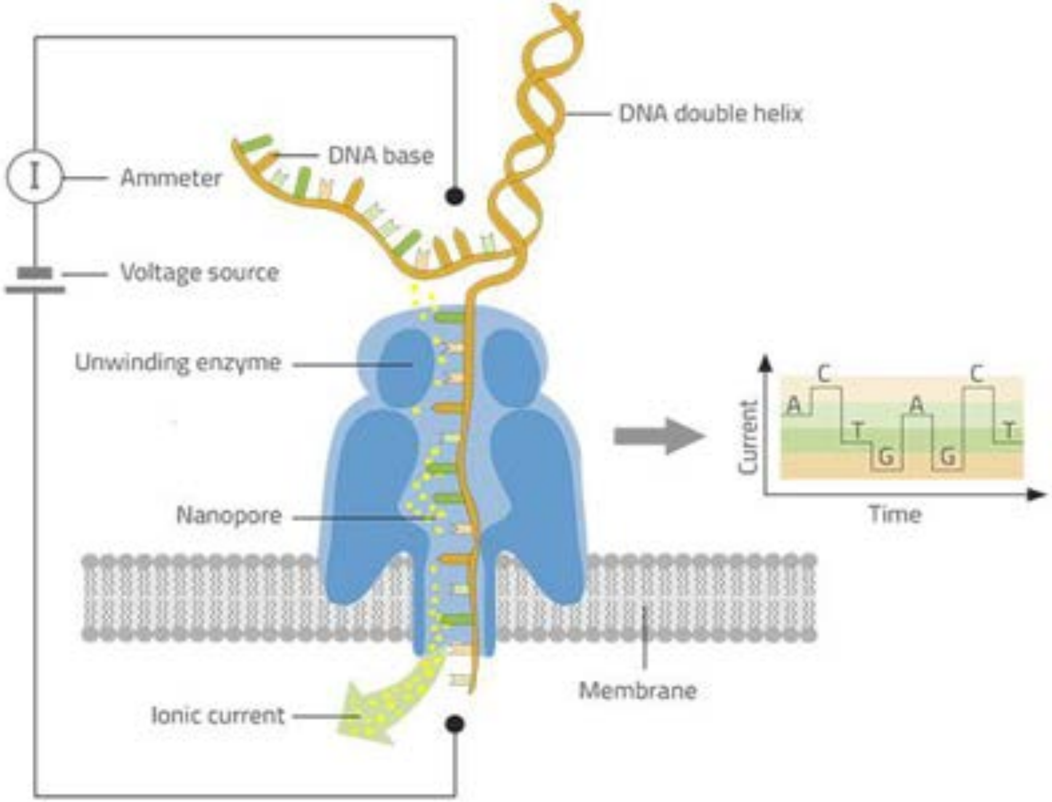
1. Overlapping PCR amplicons spanning SAV genome.
Primers in conserved regions, agnostic to sub-type variation



2. Rapid & cheap Oxford Nanopore MinION sequencing


Oliver Eve


247 Sequencing
223 Pore
10 Recovering
19 Inactive
13 Unclassified

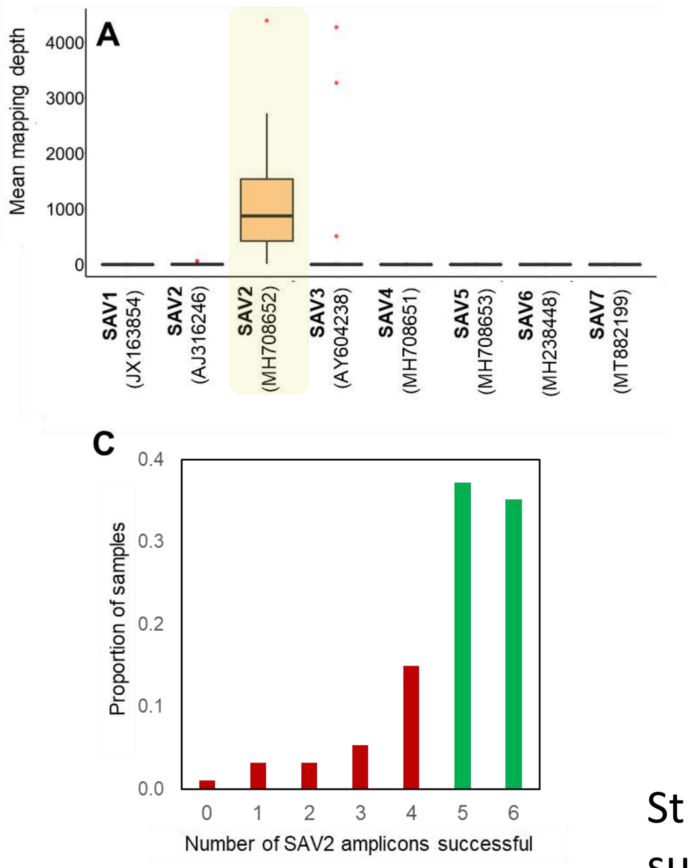


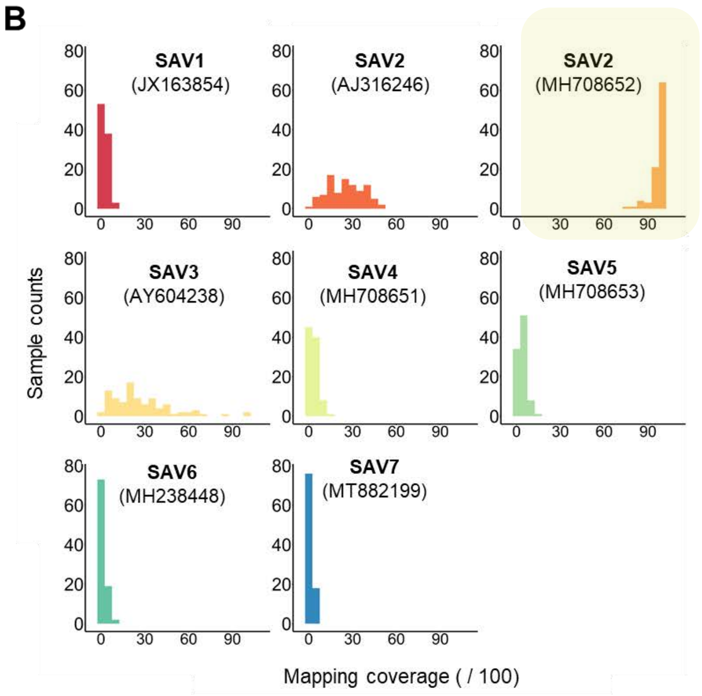
3. Bioinformatic analysis & epidemiological inferences
Strategy captures any or multiple subtypes present per sample

Bioinformatics – data captured

After filtering, we kept 68 samples for further analysis:

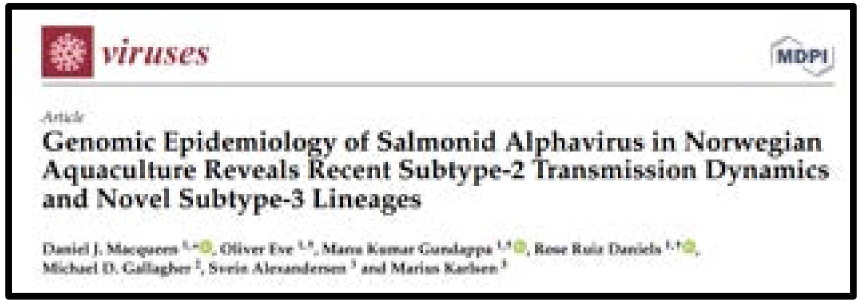
- Most sequences SAV2, as expected - mean 10,600 bp per sample, 90% of genome





Strategy captures any or multiple subtypes present per sample

Huge boost in SAV2 sequence data in public archives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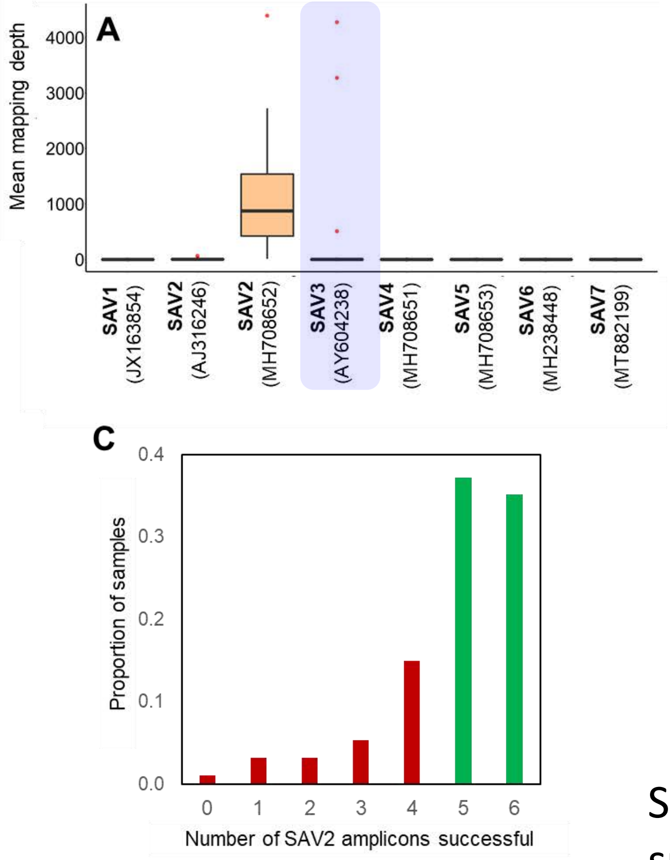
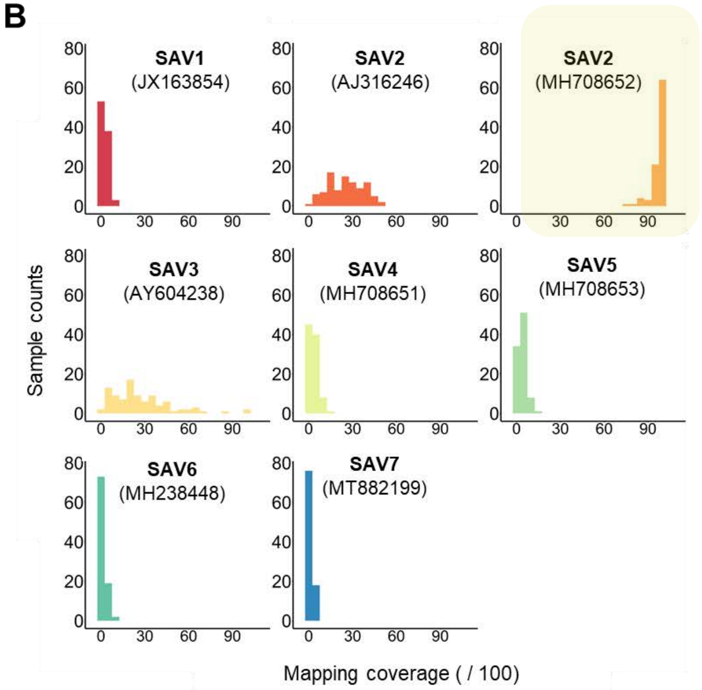
Bioinformatics – data captured

After filtering, we kept 68 samples for further analysis:

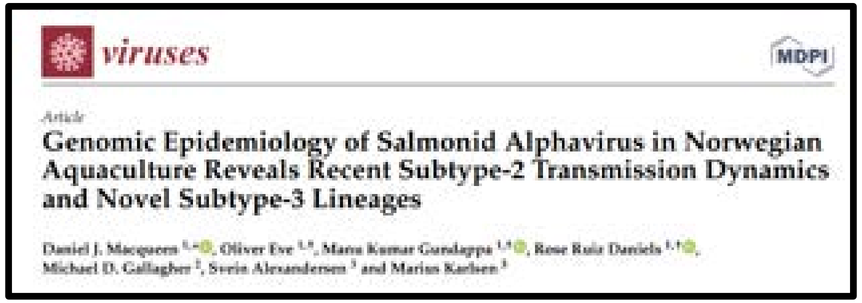
- For a few samples we captured complete SAV3 genomes (will return to this)





Strategy captures any or multiple subtypes present per sample

SAV2 data used in genomic epidemiology analyses aiming to reconstruct evolutionary history of SAV2 & viral transmission in time and space


viruses MDPI

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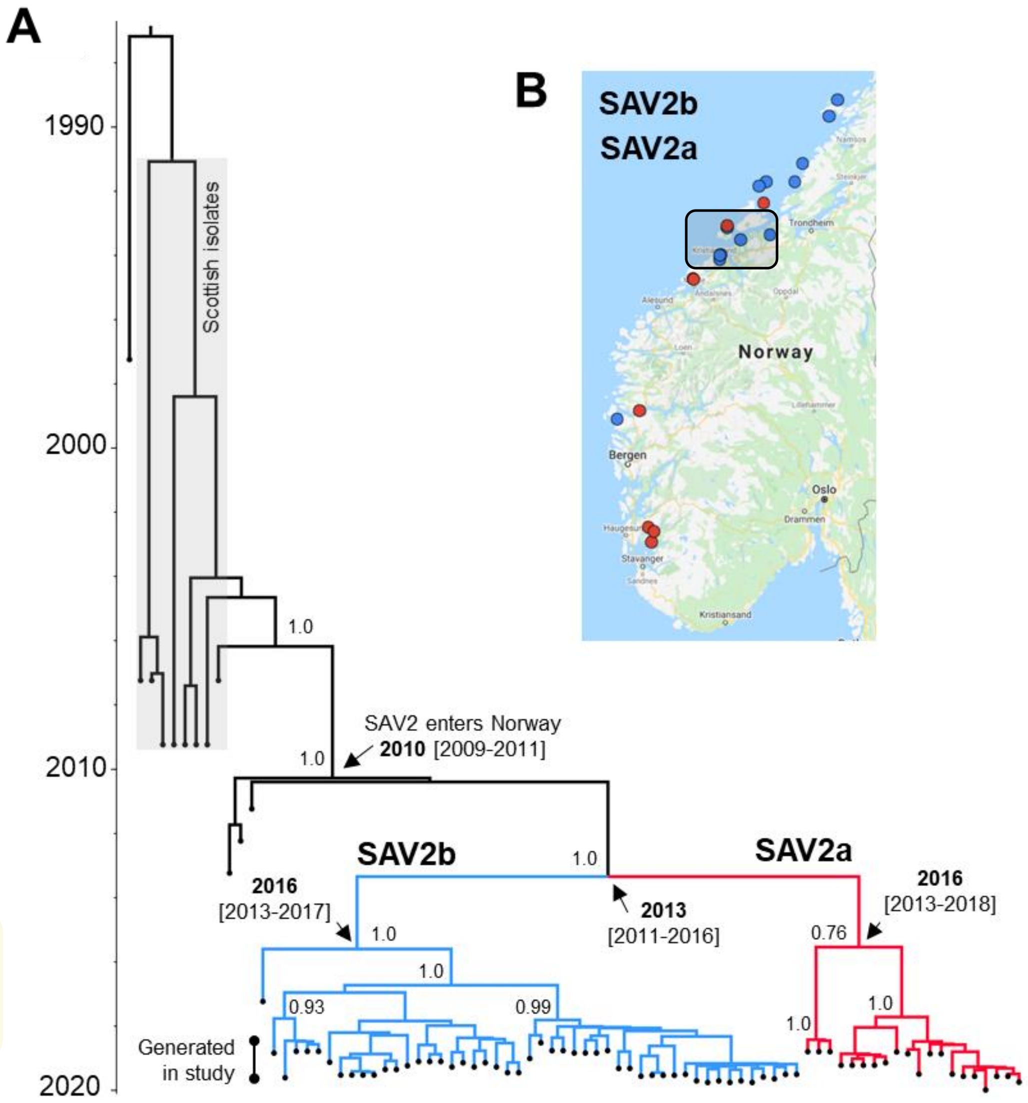
Two major SAV2 strains in Norway

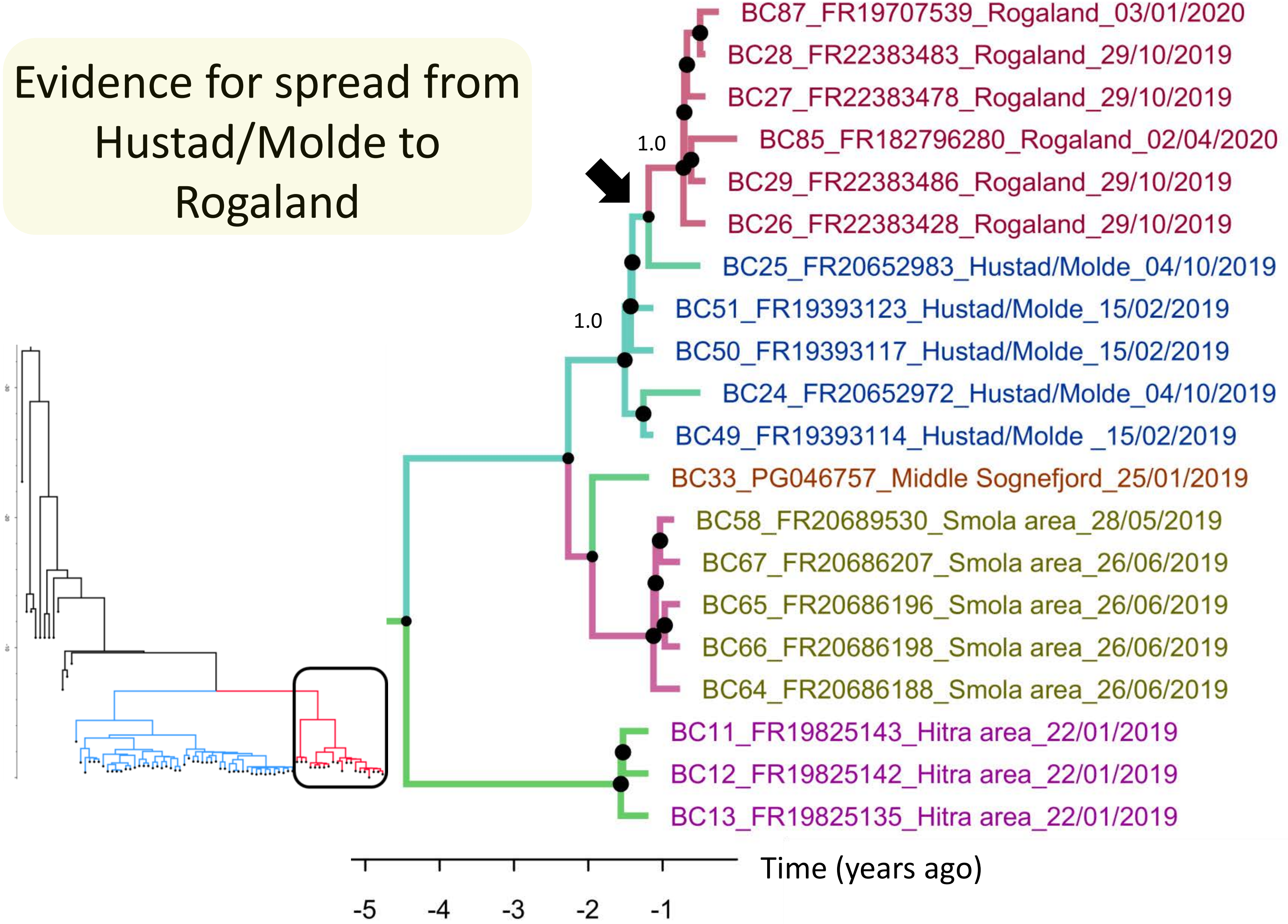
- Most recent common ancestor of Norwegian SAV2 endemic existed in ~2010
    - ➢ Probable first entry of SAV2 to Norway, but could have occurred earlier
- SAV2a & 2b likely diverged ~2013
- Largely non-overlapping detections in more Southern (2a) and Northern latitudes (2b)
    - ➢ Co-detected on same farms at boundary of Møre og Romsdal & Trøndelag

Inferences presented not possible using 'classic' approach of sequencing one viral region - insufficient phylogenetic signal



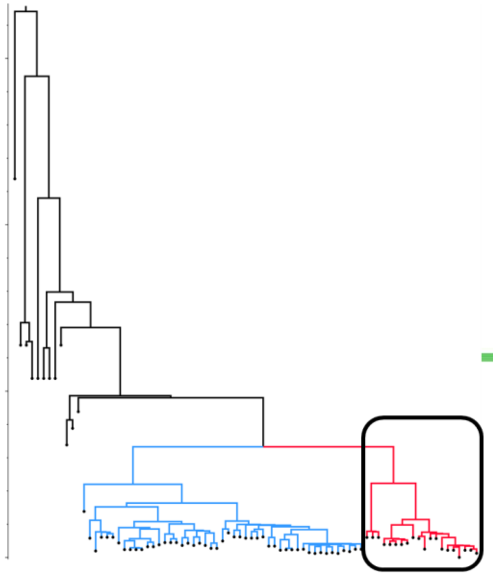
SAV2a transmission dynamics

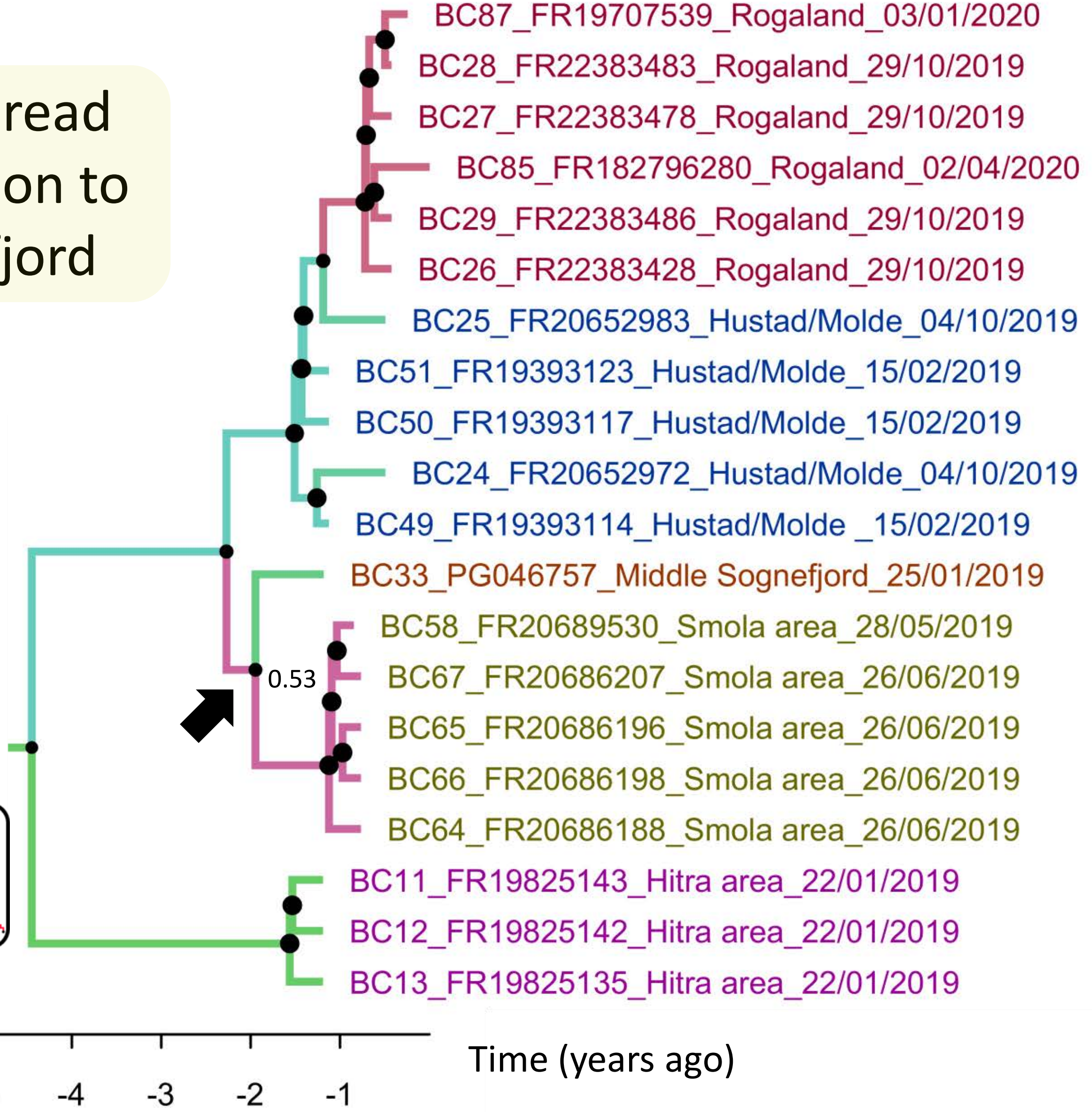
Evidence for spread from Hustad/Molde to Rogaland



SAV2a transmission dynamics

Evidence for spread from Smola region to Middle Sognefjord

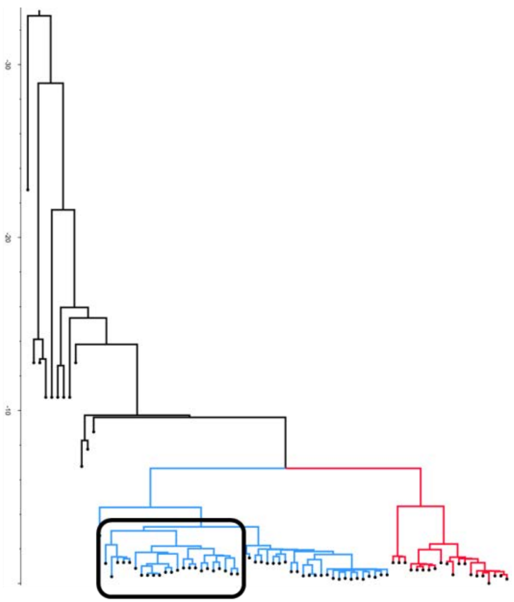


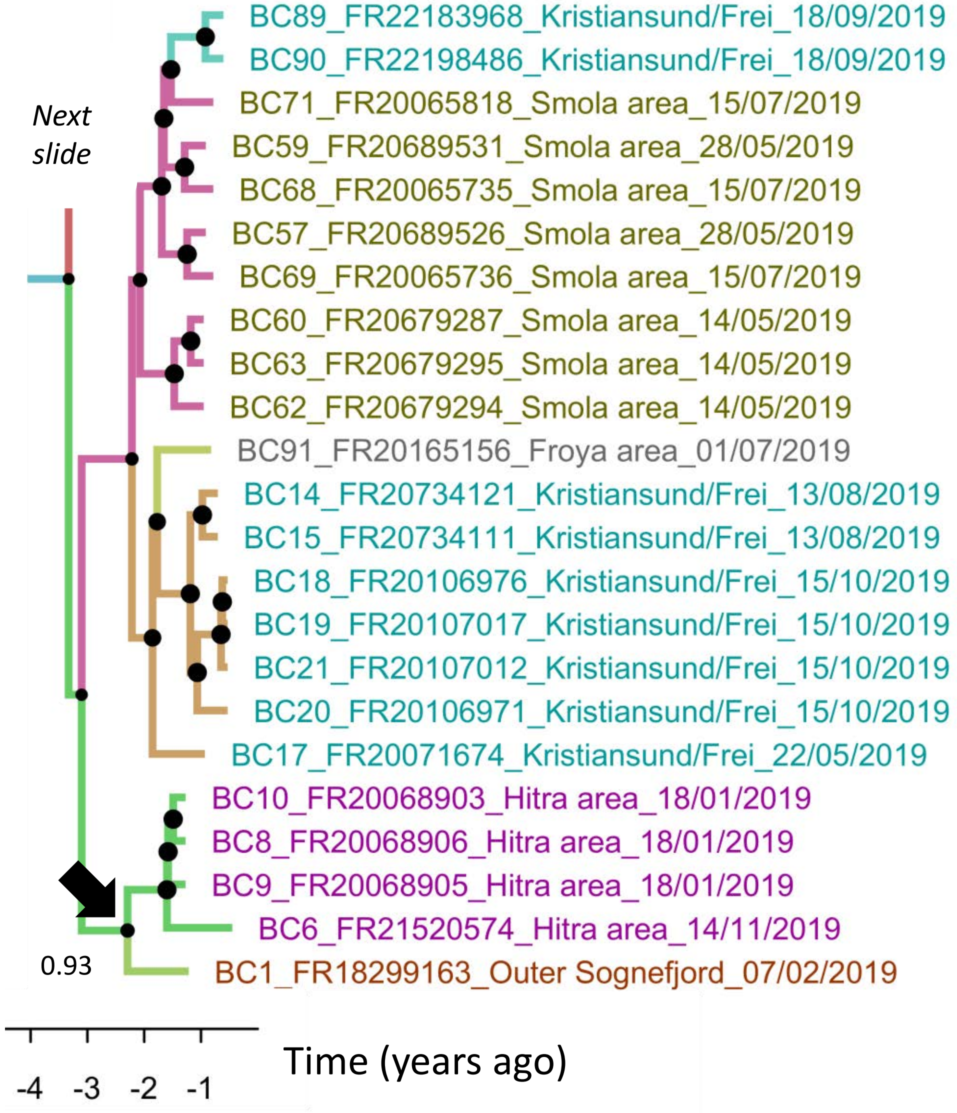


No connection to outer Sognefjord

SAV2b transmission dynamics

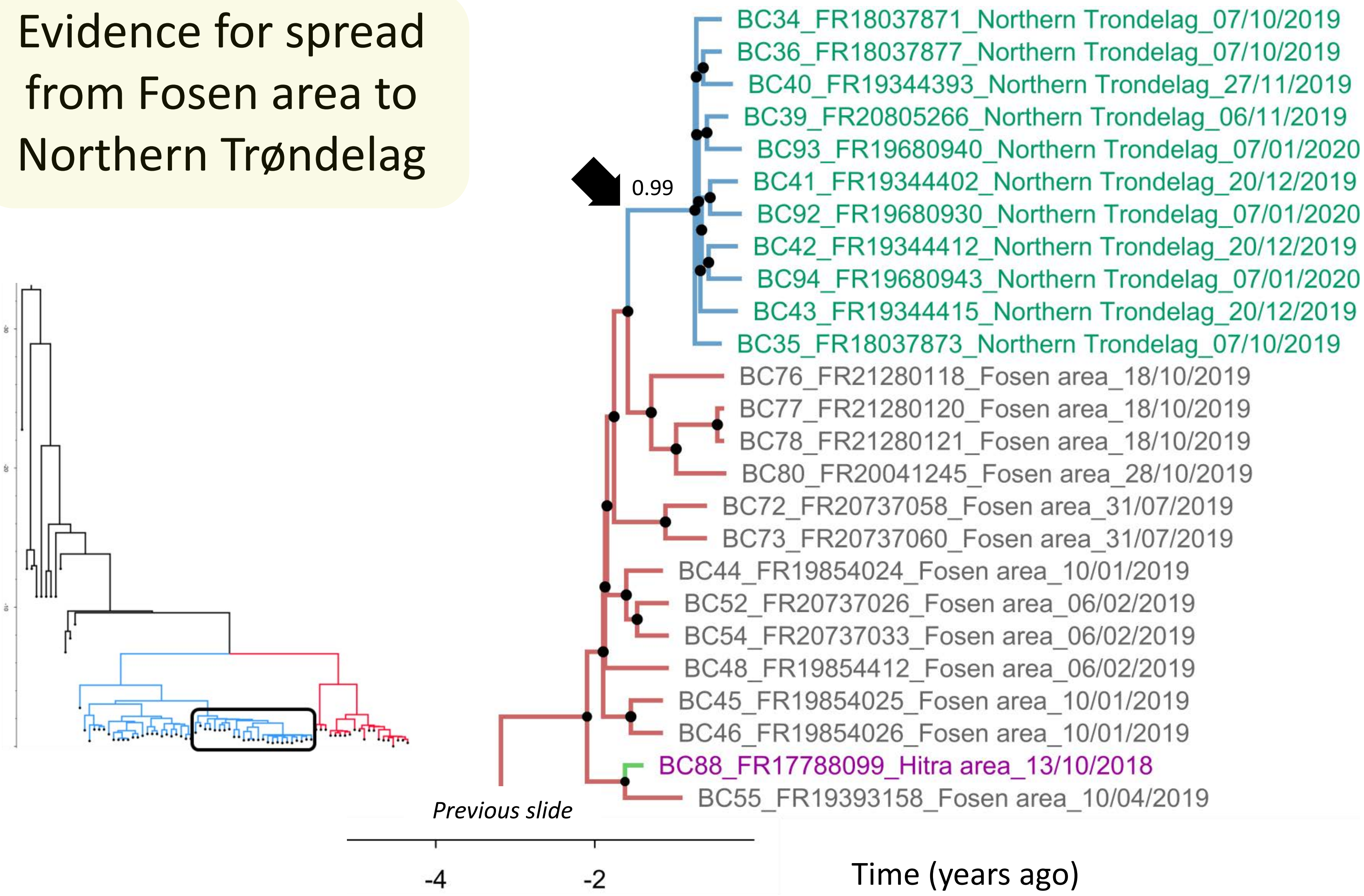
Connection between Hitra & Outer Sognefjord





SAV2b transmission dynamics

Evidence for spread from Fosen area to Northern Trøndelag



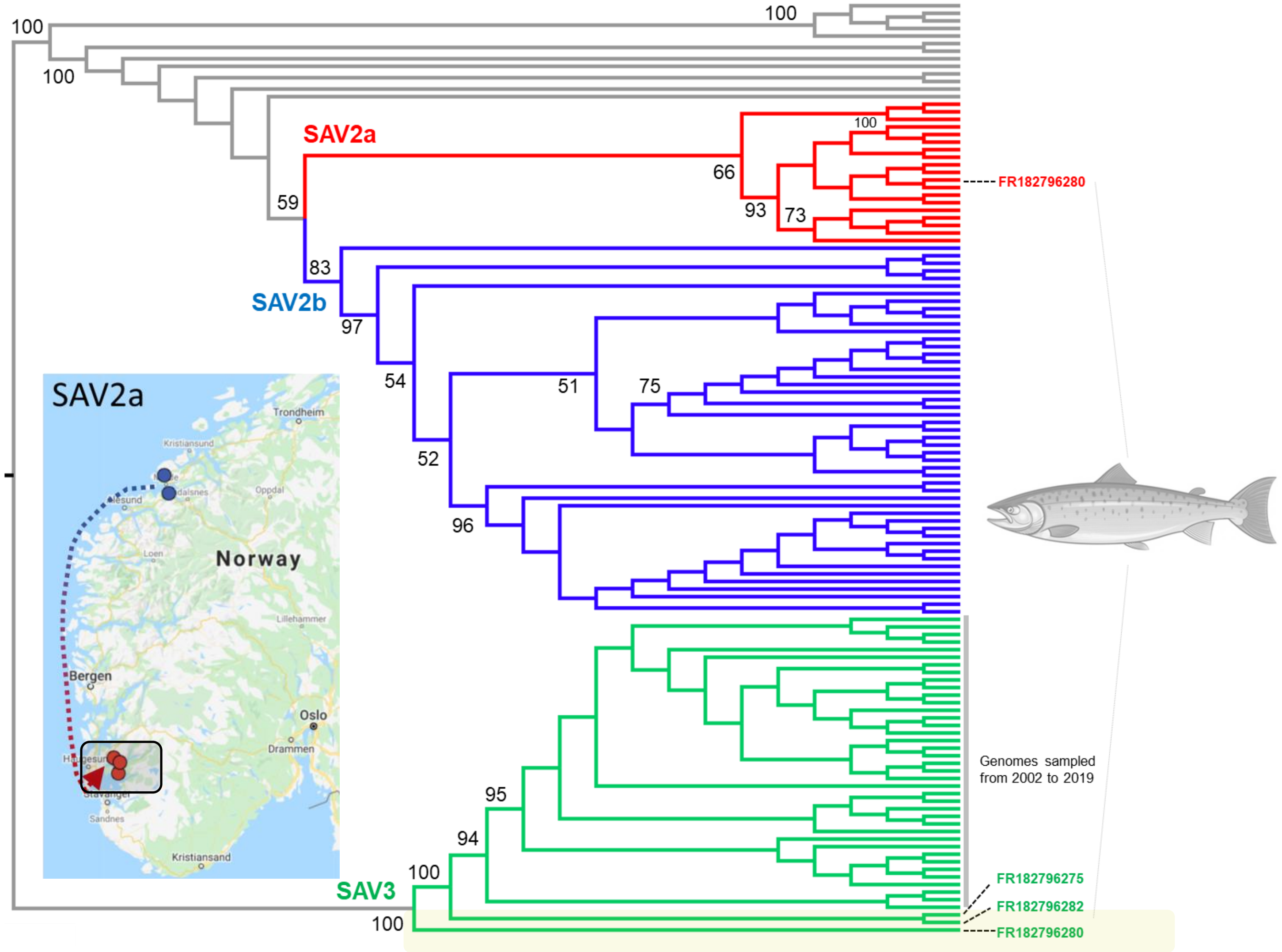
SAV2 and SAV3 co-infections

- Full genome for SAV2a & SAV3 from single samples in Rogaland

  SAV2-SAV3 co-infections arise quickly when conditions allow

- Co-infections add complexity to diagnostics & disease transmission inferences

Uncharacterized ‘ancient’ SAV3 strains – much to learn, demanding more work



Take home messages

- Validation of viral pathogen genomic sequencing for epidemiological inferences – information above & beyond current best molecular surveillance efforts
- Anthropogenic routes of disease transmission can be accounted for in mitigation planning and regulatory actions
- Cost effective & fast – easily up-scaled and transferred to other viruses

Genomic surveillance covering all the major viral pathogens could readily become a routine pillar of aquaculture disease management

New projects on viral sequencing

PhD studentship – 2022-2026 **Cross-national genomic surveillance of viral pathogens to support disease control in Atlantic salmon aquaculture** co-funded by Pharmaq & Sustainable Aquaculture Innovation Centre

- Bertie Knight just started at Roslin Institute - Oct 2022

Developing high-throughput assays to sequence genomes of SAV, PRV and IPNV from 100s of field infections sampled from **Norway & UK**

- Generate databases of genome sequences for each virus to catalogue genetic diversity and contemporary viral transmission pathways
- Aligned to live BBSRC project between Royal Veterinary College (Sarah Hill's group) with similar objectives for PMCV & ISAV
- **Please talk to us if interested or would like to be involved!**

Funding for SAV2 study: Pharmaq AS, Sinkaberg Hansen, Emilsen Fisk AS, Grieg Seafood ASA, Måsøval, Midt Norsk Havbruk, Osland Havbruk AS, Salmar

Colleagues contributed to work:
Dr Rose Ruiz Daniels, Mr Oliver Eve, Dr Manu Kumar Gundappa

Macqueen lab

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Biotechnology and Biological Sciences Research Council

eastbio

marine scotland