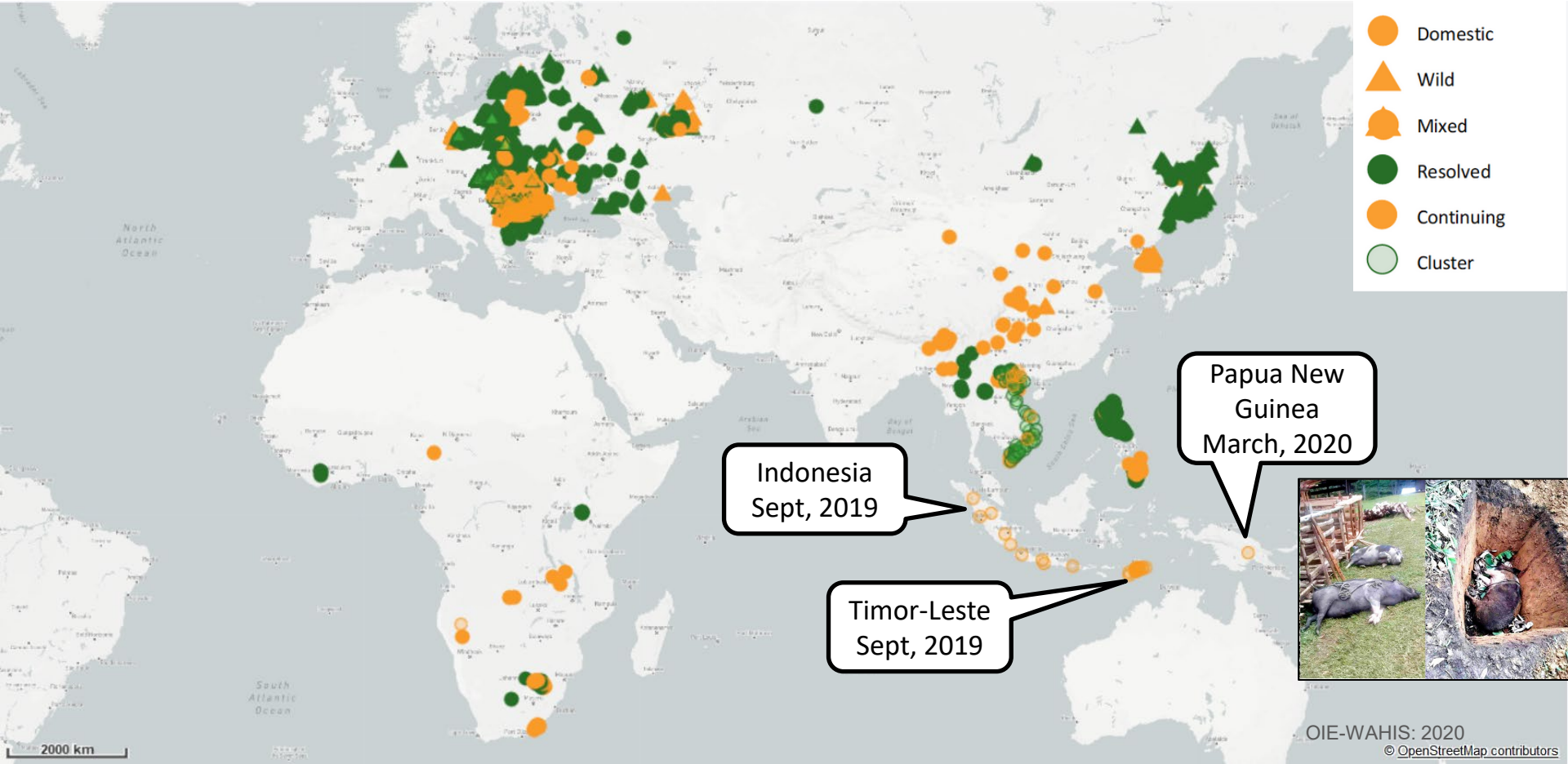
# Activities of the WOAH Reference Laboratory for ASF at the Australian Centre for Disease Preparedness (ACDP)

David Williams

4th Consultation Meeting on ASF for Southeast Asia, Hanoi, Vietnam, 26th-28th Nov 2024

# ASF: on Australia's doorstep

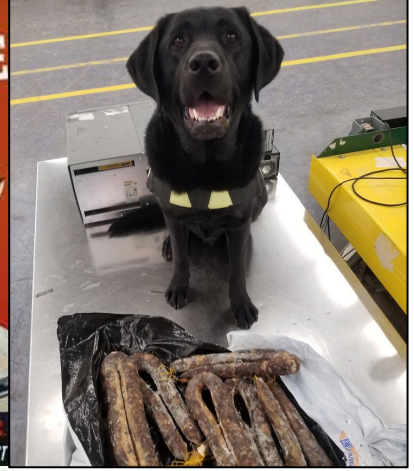


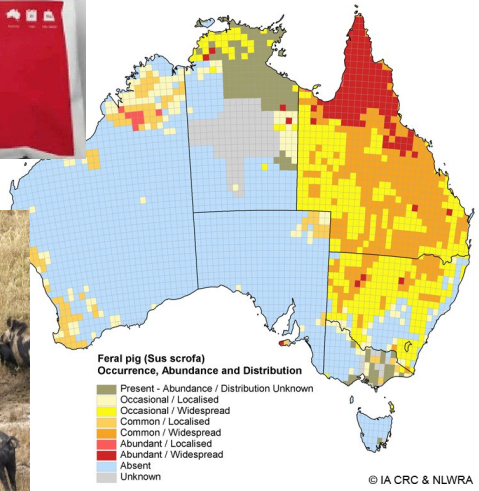
# ASF threat to Australia

- Potential mechanisms of introduction
  - Infected pork and pork products
  - Contaminated porcine genetic material
  - Contaminated equipment and clothing
  - Infected pigs or carcasses of infected pigs
  - Food waste from unregulated foreign boats and shipping in Northern Australia

→swill feeding to/contact with domestic pigs
→eaten by free-range or feral pigs

- *Large feral pig population is potential reservoir*

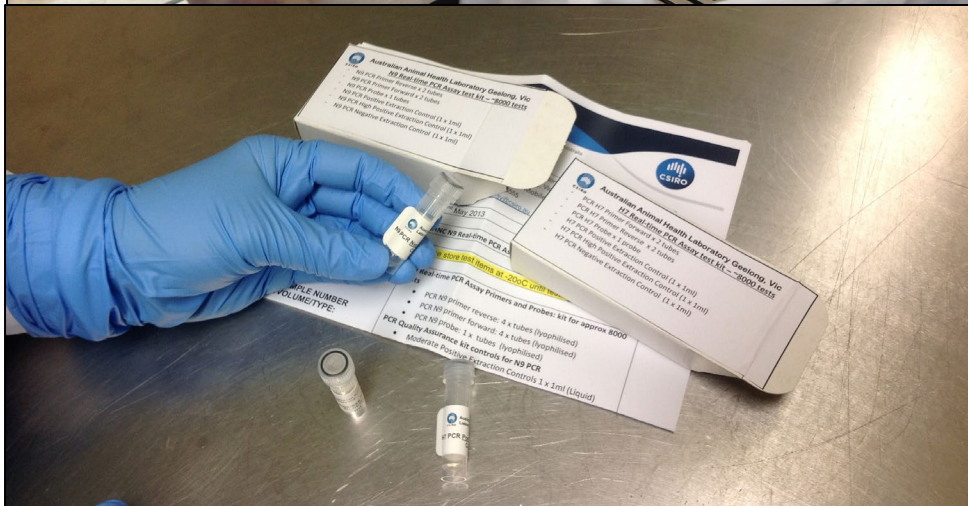



# Laboratory diagnosis at ACDP

- Multidisciplinary diagnostic capability
  - Molecular (PCR and sequencing), virology, serology and pathology
- Diagnostic or confirmatory testing and virus characterisation for outbreak response and surveillance
  - Vietnam, Myanmar, Timor Leste, Papua New Guinea (PNG), Bhutan, Nepal
  - ACDP provides diagnostic and sequence analysis report
- Diagnostic capacity building for regional animal health labs
- Testing of illegally imported pork for Australian Department of Agriculture and other countries on request

# Lab diagnostics

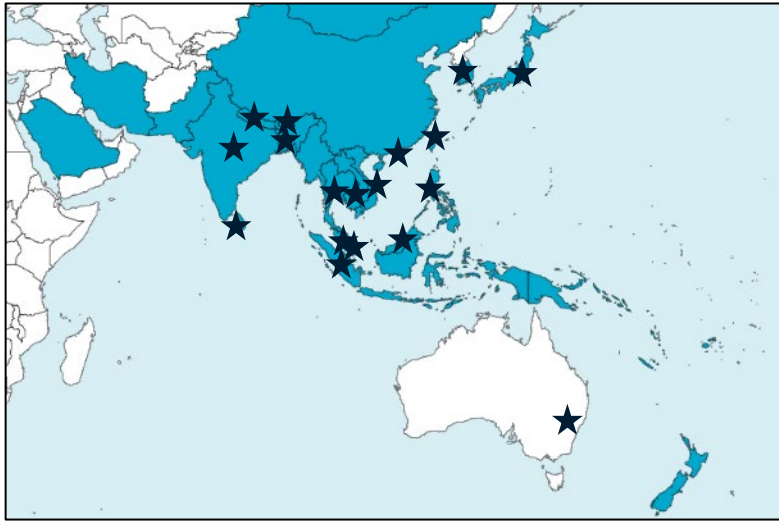
- Training and advice for:
  - Diagnostic testing (WOAH, FAO)
  - Biosafety and biosecurity
  - Sampling, packaging and shipping
- Provide diagnostic reagents (PCR kits, pos controls)
- <u>Proficiency testing (PT):</u> ISO/IEC 17043-accredited PT provider, coordinate a regional (Asia-Pacific) programme for swine disease, including ASF and CSF viruses



# ACDP Proficiency Testing: swine diseases PCR panel

- Distributed 27 PT panels, with testing conducted in June
- Panel comprised 19 samples, including ASFV, CSFV, Influenza A, PRRSV
- 18 participating countries (no. labs):

  Australia (1), Bangladesh (1), Bhutan (1), Brunei (1), Cambodia (2), Hong Kong (1), India (1), Indonesia (4), Japan (1), Korea (1), Malaysia (1), Nepal (1), Philippines (2), Singapore (1), Sri Lanka (1), Chinese Taipei (1), Thailand (3), Vietnam (8)
- Overall, the majority of laboratories returned **acceptable results for the ASF component** of the PT panel, demonstrating <u>reliable diagnostic performance</u>



# Support for Pacific Islands Countries and Indonesia for ASF Laboratory and Field Testing

- ACDP working with Papua New Guinea National Agriculture & Quarantine Inspection Authority (NAQIA) on Laboratory Capacity Building, via 4-year project
  - Priority EADs, including ASF
  - Laboratory diagnostics (focus on PCR), biosafety and quality assurance
- Previously provided training workshops and advice for use of field-testing Indonesia and Pacific Islands Countries

# Asia Pacific Placements and Laboratory Leadership (APPLLe) Project

Australian Government
Department of Foreign Affairs and Trade

- DFAT-funded project under the Partnership for a Healthy Region initiative (2023-2027)
- Can support activities throughout the Asia Pacific region with an emphasis on developing technical and leadership capability within laboratories in **Vietnam** to enable better representation in global disease networks

*Contact: Jemma.Bergfeld@csiro.au*

- Activities include:
  - Laboratory placements (at ACDP or a leading laboratory within SE Asia)
  - Technical training and workshops led by ACDP staff for high priority TADs
  - Strengthening diagnostic expertise for **ASF** at RAHO6 in Ho Chi Minh City
  - Development of sequencing capability (via MinION), particularly for **ASF**
  - Co-facilitation of workshops with ACDP for other laboratories in the region
  - Development of testing protocols for investigation of new and emerging diseases at the human-animal-wildlife interface

# Protocols and Guidelines for Laboratory Diagnosis

- Addresses detection of:
  - Virulent and variant forms of ASFV
  - LAVs using DIVA PCRs
  - Technical procedures and guidance
- Developed in consultation with the **WOAH ASF Reference Laboratory Network**, based on WOAH recommendations for laboratory diagnosis of ASF
- *Available online*

Addressing African Swine Fever

Protocols and Guidelines for Laboratory Diagnosis

January 2024

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The World Organisation for Animal Health (WOAH), the Food and Agriculture Organization of the United Nations (FAO) and other partners have been actively working in countries affected or at risk of incursion by African swine fever (ASF). This manual is an updated and expanded version of guidance first published in 2020 following the emergence of ASF in China, other Asian countries, and countries of the Pacific and Caribbean regions.

World Organisation for Animal Health
Founded as OIE

www.woah.org/en/disease/african-swine-fever/

# ASF molecular typing and epidemiology

- Determine genotypes of circulating viruses and genetic variation within and between populations
    - Can be used to identify recombinants

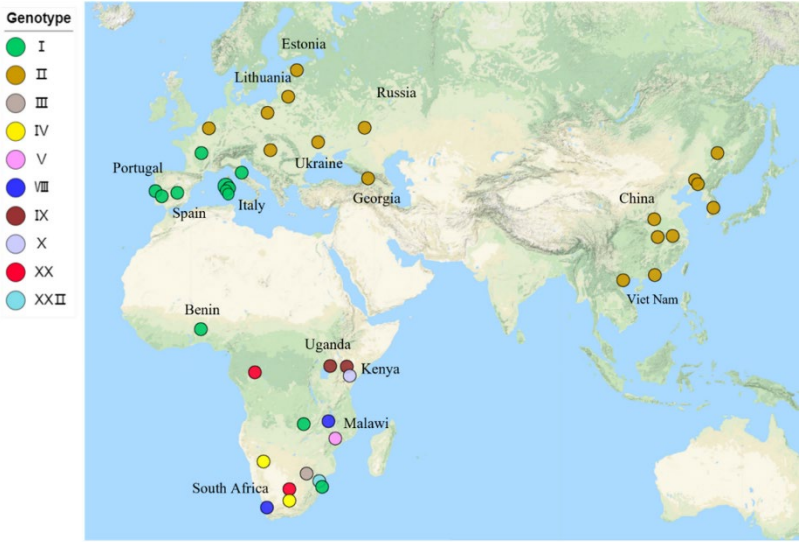
- Patterns of distribution and spread can be determined by combining surveillance data with genetic data

- Used for trace forward/trace back investigations during outbreaks, understand source and origin

- Important for vaccine matching

# Molecular typing and epidemiology

- Several genes and combinations have been used
  - Partial p72 gene for genotyping, based on Bastos et al. (2003)
    - → 24 genotypes
    - → Only two found outside Africa
- Other targets: Central variable region of *B602L*(9RL), *KP86R*, *E183L* (p54) and *CP204L* (p30), *I73R-I329L* intergenic region (IGR)




Shen et al. 2022. doi.org/10.3390/v14050889

IGR:

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Georgia 2007/01  172351 ACAAGTATATAGGAATATATAGGAATATATA----------GAAATATATAGAAATAGCTAAGCT
ASFV China 2018/     81 ACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCT
Pol14/Krus           81 ACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCT
Bel13/Grodno         81 ACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCT
Ukr12/Zapo           81 ACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCT
Lt14/1490            81 ACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCT
Lt14/1482            81 ACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCT
Pol14/Sz             81 ACAAGTATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCT
Armenia/07           81 ACAAGTATATAGGAATATATAGGAATATATA----------GAAATATATAGAAATAGCTAAGCT
Tver0511/Torjo       81 ACAAGTATATAGGAATATATAGGAATATATA----------GAAATATATAGAAATAGCTAAGCT
Rostov09             81 ACAAGTATATAGGAATATATAGGAATATATA----------GAAATATATAGAAATAGCTAAGCT
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# Recent Developments for ASF typing

- Full length p72 protein sequence
  - ‘proteotype’
  - 6 new p72 groups proposed:

| New genotype/ proteotype | Original genotype *(partial p72 gene)* |
|---|---|
| 1 | I, VII, XXII |
| 2 | II, III, IV, V, XVII, XX, XXI, XXIV |
| 8 | VIII |
| 9 | IX, X |
| 15 | XV |
| 23 | XXIIIa, XXIIIb |

*viruses* MDPI

*Article*

**A Re-Evaluation of African Swine Fever Genotypes Based on p72 Sequences Reveals the Existence of Only Six Distinct p72 Groups**

Edward Spinard [1,2,3], Mark Dinhobl [1,2,3], Nicolas Tesler [1,4], Hillary Birtley [1,4], Anthony V. Signore [3,5], Aruna Ambagala [3,5], Charles Masembe [3,6], Manuel V. Borca [1,2,3,*] and Douglas P. Gladue [1,2,3,*]

[1] Plum Island Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture,

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viruses MDPI

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AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

| Virology | Announcement

**African swine fever virus P72 genotyping tool**

Mark Dinhobl,[1,2,3] Edward Spinard,[1,2,3] Hillary Birtley,[2,3,4] Nicolas Tesler,[2,3,4] Manuel V. Borca,[1,2,3] Douglas P. Gladue[1,2,3]

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT Historically, genotyping of African swine fever virus was based on partial sequencing of B646L (p72). Until recently, the number of differences that defined genotypes was ambiguous. This tool allows a sequence to be uploaded and will report its closest matches along with its likely p72 genotype.

*asfvgenomics.com*

# Recent Developments for ASF typing

- Analysis of complete protein coding sequence (*proteome*) of ASFV genome derived from whole genomes → 7 **biotypes** proposed:

viruses MDPI

*Article*

**Reclassification of ASFV into 7 Biotypes Using Unsupervised Machine Learning**

Mark Dinhobl [1,2,3,†], Edward Spinard [1,2,3,†], Nicolas Tesler [1,4], Hillary Birtley [1,4], Anthony Signore [3,5], Aruna Ambagala [3,5], Charles Masembe [3,6], Manuel V. Borca [1,2,3,*] and Douglas P. Gladue [1,2,3,*]

[1] United States Department of Agriculture, Agricultural Research Service, Foreign Animal Disease Research

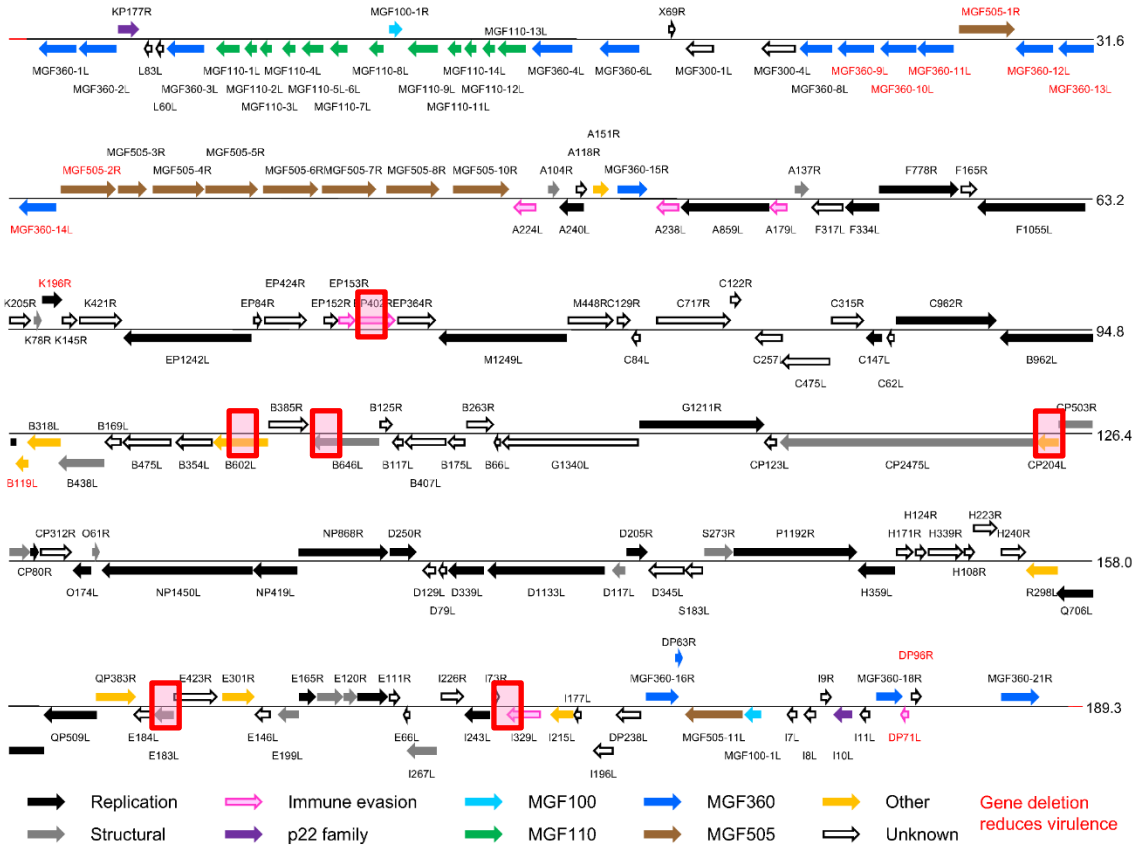
| Biotype | New genotype/ proteotype | Original genotype *(partial p72 gene)* |
|---|---|---|
| 1 | 1 | I, VII |
| 1/2 R* | 2 | II |
| 2 | 2 | II |
| 3 | 2 | III, IV, V, XX |
| 4 | 9 | X |
| 5 | 9 | IX |
| 6 | 8, 15 | VIII, XV |

**1/2 R is comprised of recombinants genomes of biotypes 1 and 2*

# A role for whole genome sequencing (WGS) for molecular epidemiology?

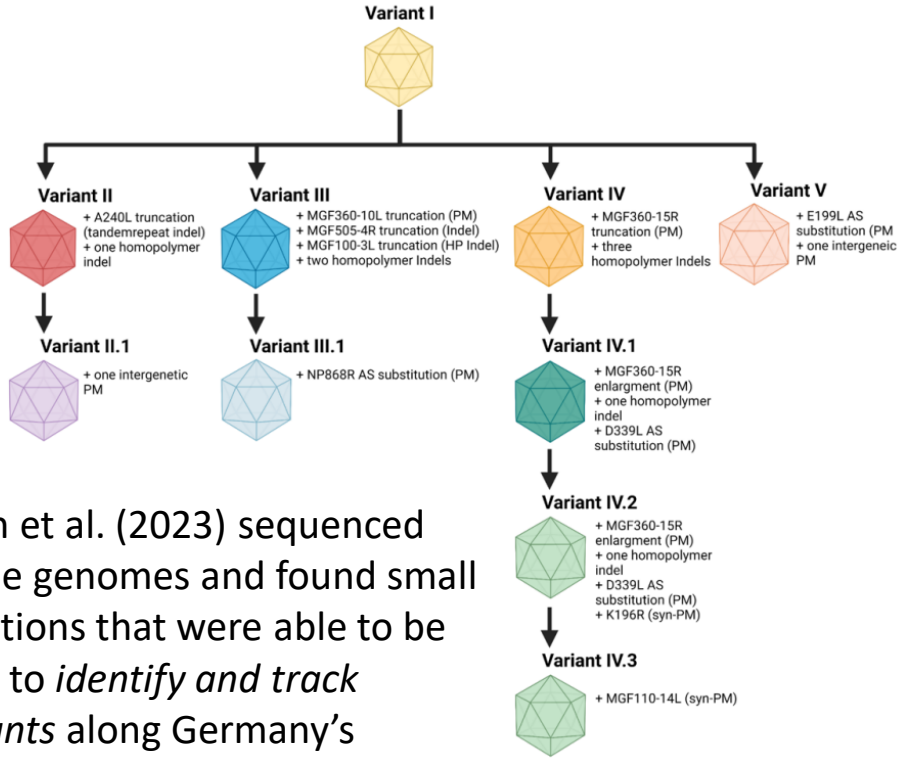
The ASFV genome is very large and highly stable. Individual gene targets ( ) are of limited use for the identification of variants and the application of phylogeny for studying evolution

*WGS are increasingly being used for these purposes*

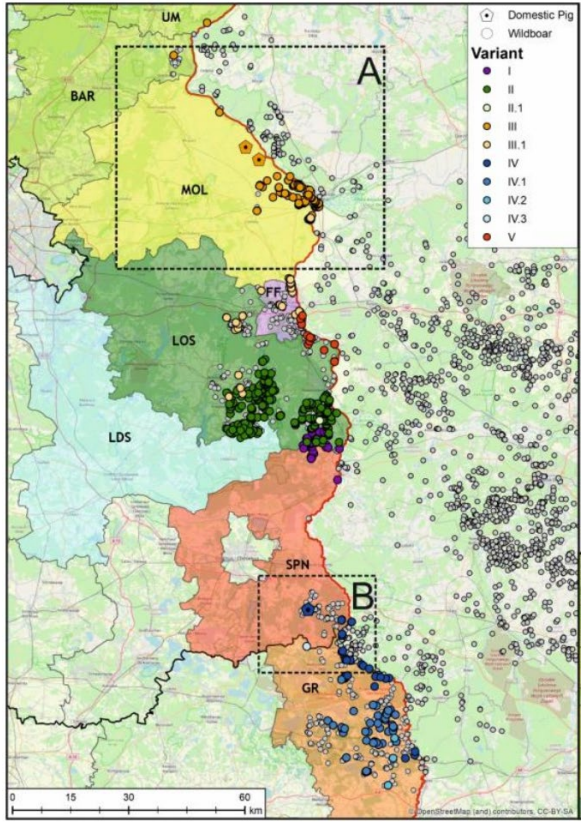



*Alonso* et al. *J. Gen. Virol., 99: 613–614 (mod. From Dixon* et al. *Virus Res. 173 (1):3)*

# Whole genome molecular epidemiology: Germany



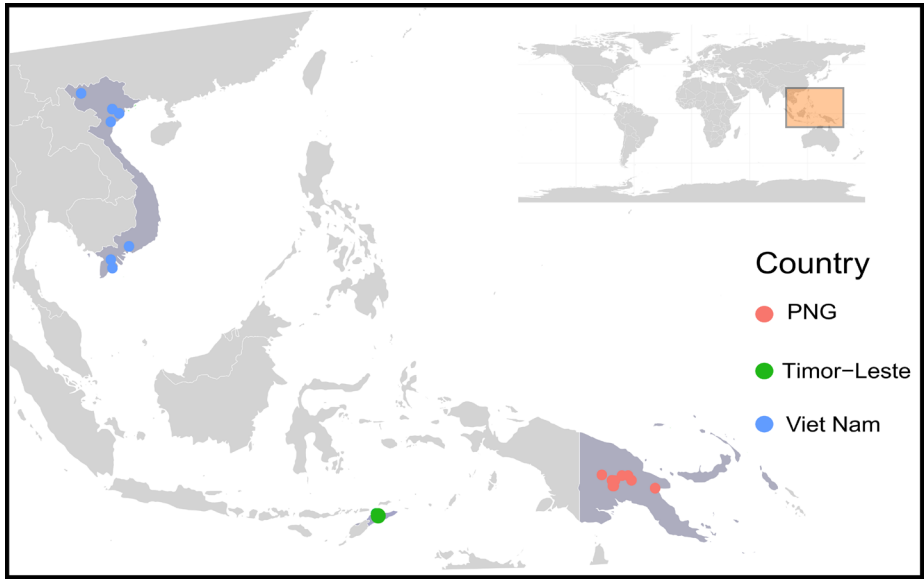

Forth et al. (2023) sequenced whole genomes and found small variations that were able to be used to *identify and track variants* along Germany's eastern border with Poland




Forth et al. 2023. https://doi.org/10.1101/2022.09.07.506908

# ASFV Genomics: Emergence of microvariants of Genotype II in the Asia-Pacific region

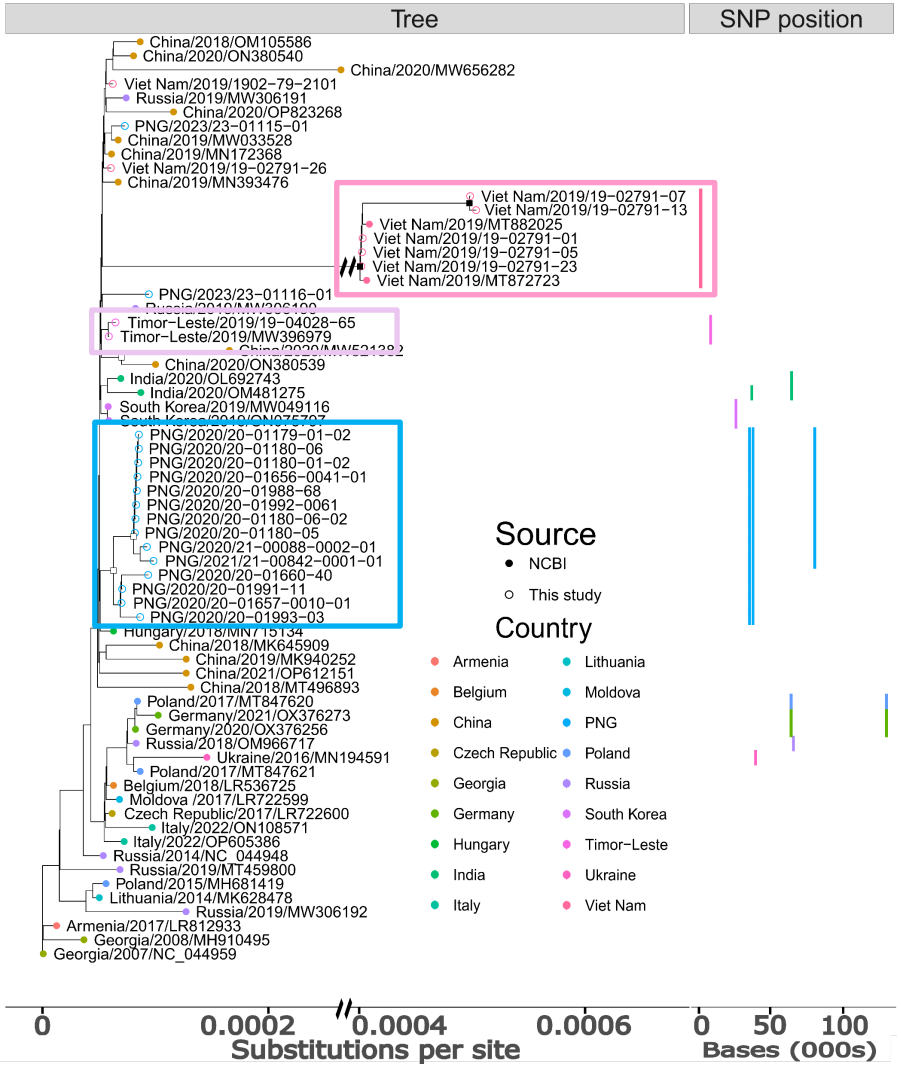
- We investigated the presence of novel mutations in ASFV whole genomes from the Asia-Pacific
  - Derived from 25 samples collected from Viet Nam, Papua New Guinea, Timor-Leste
  - Submitted to ACDP ASF Reference Laboratory
  - Assessed usefulness of ASFV WGS for molecular epidemiology




James O'Dwyer, Matthew Neave, ACDP

# Emergence of microvariants in the Asia-Pacific

- Subtle genetic differentiation was found in samples from each locality
- Multiple individual mutations were found only within specific countries
- Geographic clustering of sequences from each country in phylogenetic analysis



# Detection of Recombinant ASFV

- Emergence of **recombinant genotype I/II** reported in China, Vietnam and E. Russia associated with acute ASF
- Identification by:
  - Conventional PCR using 2 or more and sequencing *OR*
  - WGS and bioinformatics → patterns of recombination across the genome

nature communications

Article https://doi.org/10.1038/s41467-023-38868-w

**Highly lethal genotype I and II recombinant African swine fever viruses detected in pigs**

Dongming Zhao[1,2], Encheng Sun[1,2], Lianyu Huang[1,2], Leilei Ding[1,2], Yuanmao Zhu[1,2], Jiwen Zhang[1], Dongdong Shen[1], Xianfeng Zhang[1], Zhenjiang Zhang[1], Tao Ren[1], Wan Wang[1], Fang Li[1], Xijun He[1] & Zhigao Bu[1,2]

DISPATCHES

**Detection of Recombinant African Swine Fever Virus Strains of p72 Genotypes I and II in Domestic Pigs, Vietnam, 2023**

Van Phan Le, Van Tam Nguyen, Tran Bac Le, Nguyen Tuan Anh Mai, Viet Du
Thi Tam Than, Thi Ngoc Ha Lai, Ki Hyun Cho, Seong-Keun Hong, Yeon H
Tran Anh Dao Bui, Thi Lan Nguyen, Daesub Song, Aruna Ambagal

Molecular Biology Reports (2024) 51:1011
https://doi.org/10.1007/s11033-024-09961-0

BRIEF REPORT

**Detection of the first recombinant African swine fever virus (genotypes I and II) in domestic pigs in Russia**

Alexey Igolkin[1] · Ali Mazloum[1,2] · Nikolay Zinyakov[1] · Roman Chernyshev[1] · Antoinette van Schalkwyk[3,4] · Andrey Shotin[1] · Ivan Lavrentiev[1] · Konstantin Gruzdev[1] · Ilya Chvala[1]

# Which typing system is best to use?

- Single gene targets:
  - Original p72 partial gene typing offers simple and robust PCR-based method
  - Other targets provide additional levels of typing (e.g., *B602L* etc)
  - Lack sensitivity molecular epidemiology, phylogenetic analyses
- Whole genome sequencing:
  - Requires WGS and bioinformatics capability, and computing hardware
  - Sequencing from clinical samples is also technically challenging
  - WGS can provide highest resolution for molecular epidemiology and characterization of recombinant genomes

# Summary

- Continued surveillance will be essential for understanding circulating types and genomic diversity of ASFV in the region
  - Additional whole genome sequences (as well as dissemination/publication) will enable analysis of evolution at the SEA regional level
- ACDP provides support for regional countries through the ASF Reference Laboratory, including for conventional typing, WGS and bioinformatics analyses
- New ASFV typing methods offer promise, but existing typing methods likely to be used alongside

# Thank you

Dr. David Williams, Leader - Diagnosis and Mammalian Infectious Disease Research Group

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