Molecular characterization of ASF outbreaks in Spain and existing gaps

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Fundada como OIE

WEBINAR, AFRICAN SWINE FEVER IN SPAIN: AN UPDATE
15TH APRIL 2026

The confirmation of the outbreak. Collaboration between laboratories.

Passive surveillance: detection of 2 dead wild boar.

→

Samples sent to regional laboratory – PCR positive

Samples sent to the National Reference Laboratory (LCV–Algete)

Outbreak confirmation.

Notification by the competente authorities to the WOAH

↓

EURL for ASF (CISA/INIA-CSIC)

Genetic characterization of the virus responsable of the outbreak

❶ **Two DNA samples extracted from blood** sent by the NRL (1st December) to the **EURL for molecular characterization of virus responsible of the outbreak**

❷ **Clinical material** comprising e **blood, serum, spleen, tonsil, and lymph node** samples for each of the two wild boar were additionally sent to the EURL for further analysis including **determination of antibody titer and virus isolation**

Wild boar ID	Sample type	PCR, Ct	ELISA result	IPT result
WB1	Serum	26.83	Negative	Positive (1:2560)
WB1	Blood	25.80	NT	Positive (1:1280)
WB1	Spleen	20.29	NT	Weak (1:10)
WB1	Lymph node	22.54	NT	Weak (1:20)
WB1	Tonsil	23.55	NT	Weak (1:20)
WB2	Serum	23.02	Positive	Positive (1:2560)
WB2	Blood	20.81	NT	Positive (1:320)
WB2	Spleen	20.76	NT	Weak (1:20)
WB2	Lymph node	21.89	NT	Weak (1:40)
WB2	Tonsil	18.87	NT	Positive (1:160)

- The virus was isolated with **haemadsorbing pattern**
- **Antibodies detected in both animals**. Serum with antibody titers (1:2560).

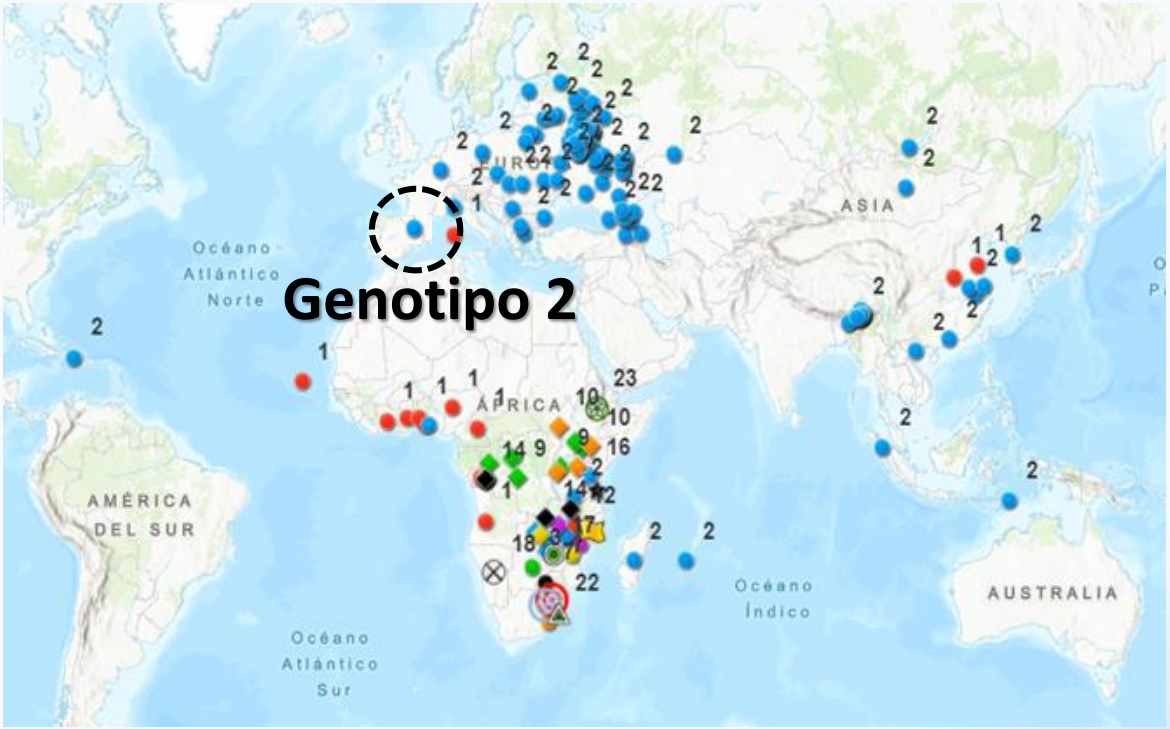
Key questions after outbreak detection:

- Where did the **virus originate**?
- How did it **reach this area**?
- Is this an **isolated event**?

In November 2025, the closest European outbreaks were >600 km away (Italy)

How do we establish genetic relationships?

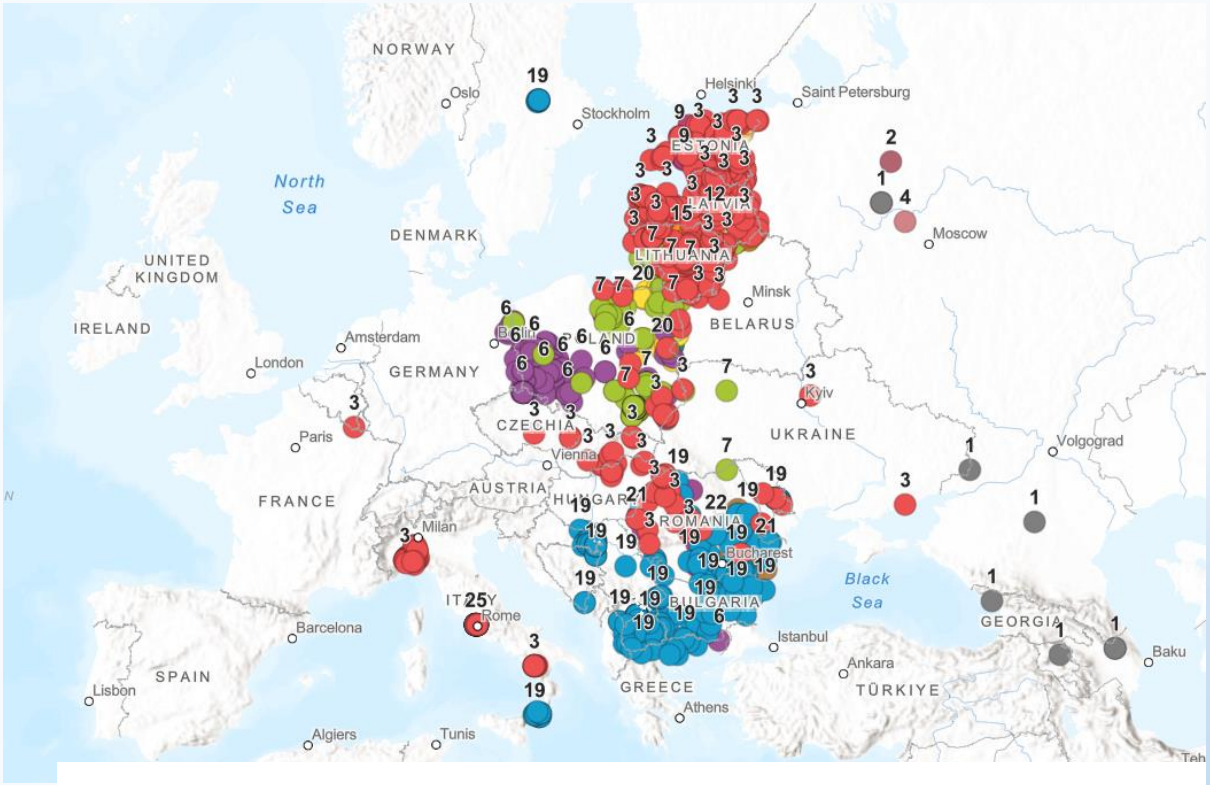
Level 1 –Global classification into one of the 24 described genotypes (C terminal end of p72 protein).



The Spanish virus is classified within genotype 2

Higher resolution is needed for tracing outbreaks

Level 2 – Multigene approach (sequencing of 6 regions) **to classify genotype 2 isolates in one of the 28 groups.**



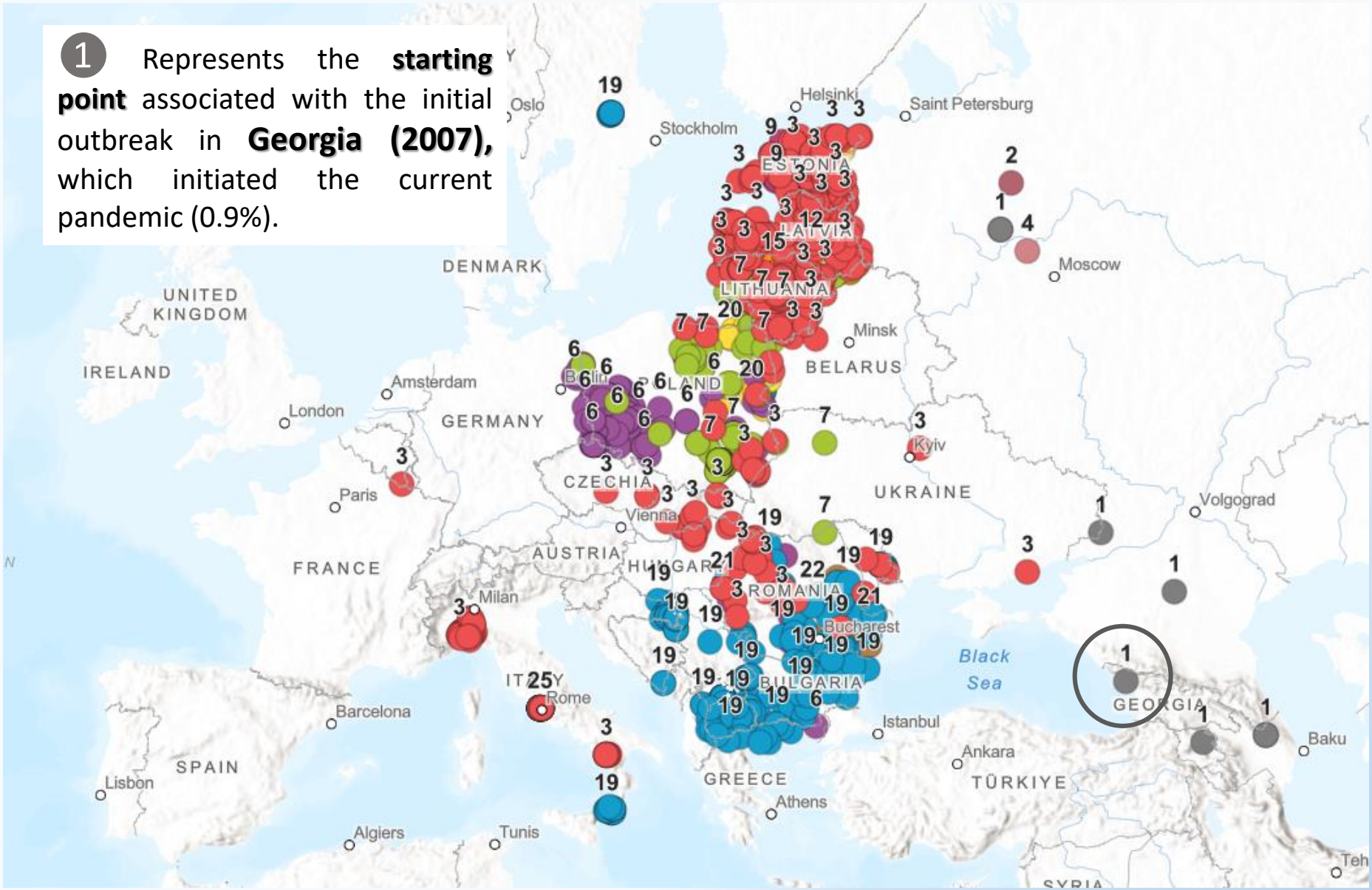
Comparison with 1,257 European ASFVs

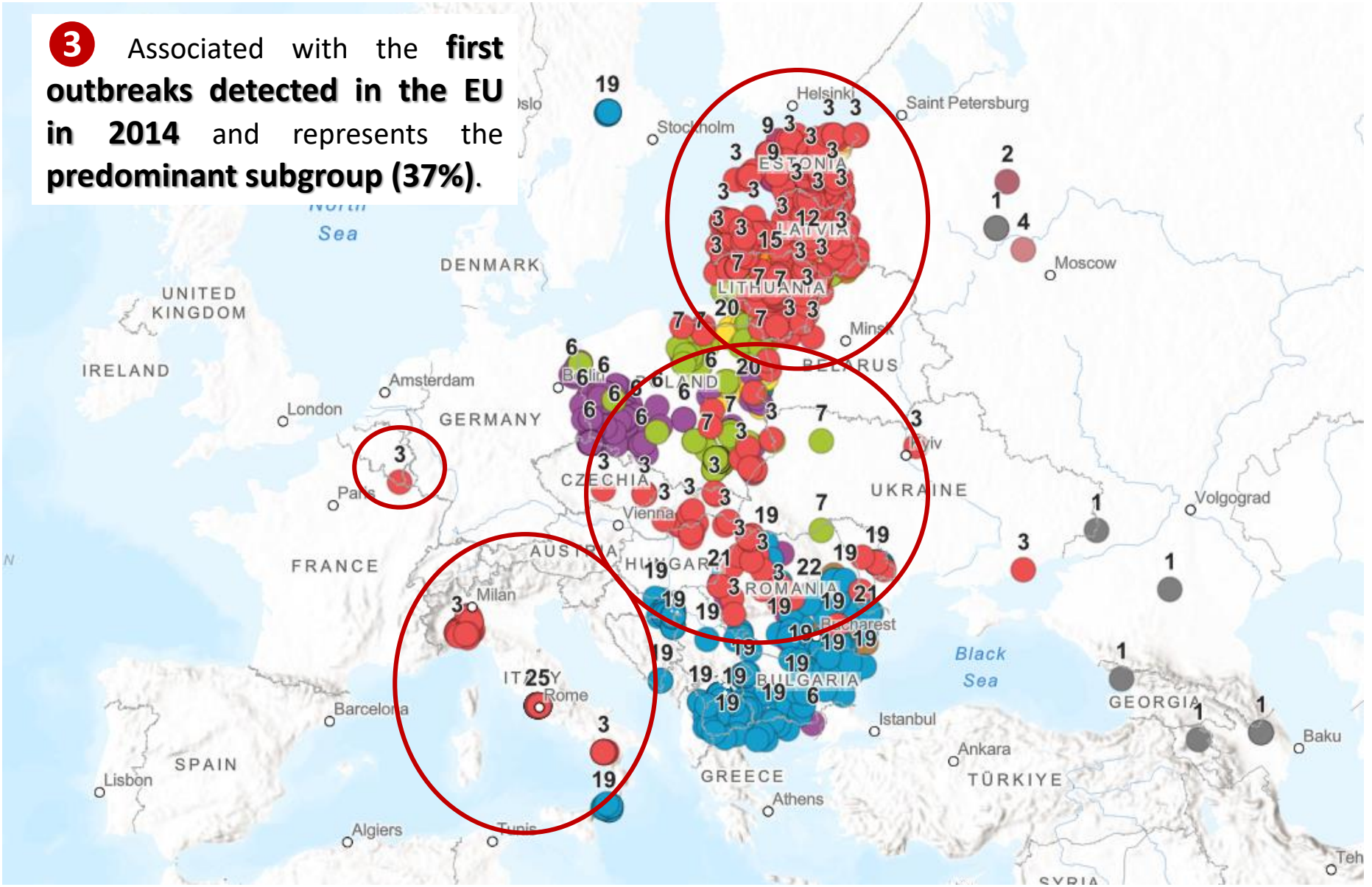
Level 2 – Genetic groups are defined by the **combination of variants across key genomic markers**

Variant / Group	CVR B602L	IGR I73R–I329L	O174L	K145R	MGF505 *9R/10R*	ECO2 I329L -I215L	Frec.
Group 1 - Original lineage – Georgia 2007	I	I	I	I	I	I	0.9%
Group 3 – dominant Europe	I	II	I	I	I	I	37%
Group 19	I	II	I	I	I	II	23%
Group 7	I	II	I	II	I	I	13%
Group 6	I	II	II	II	I	I	13%

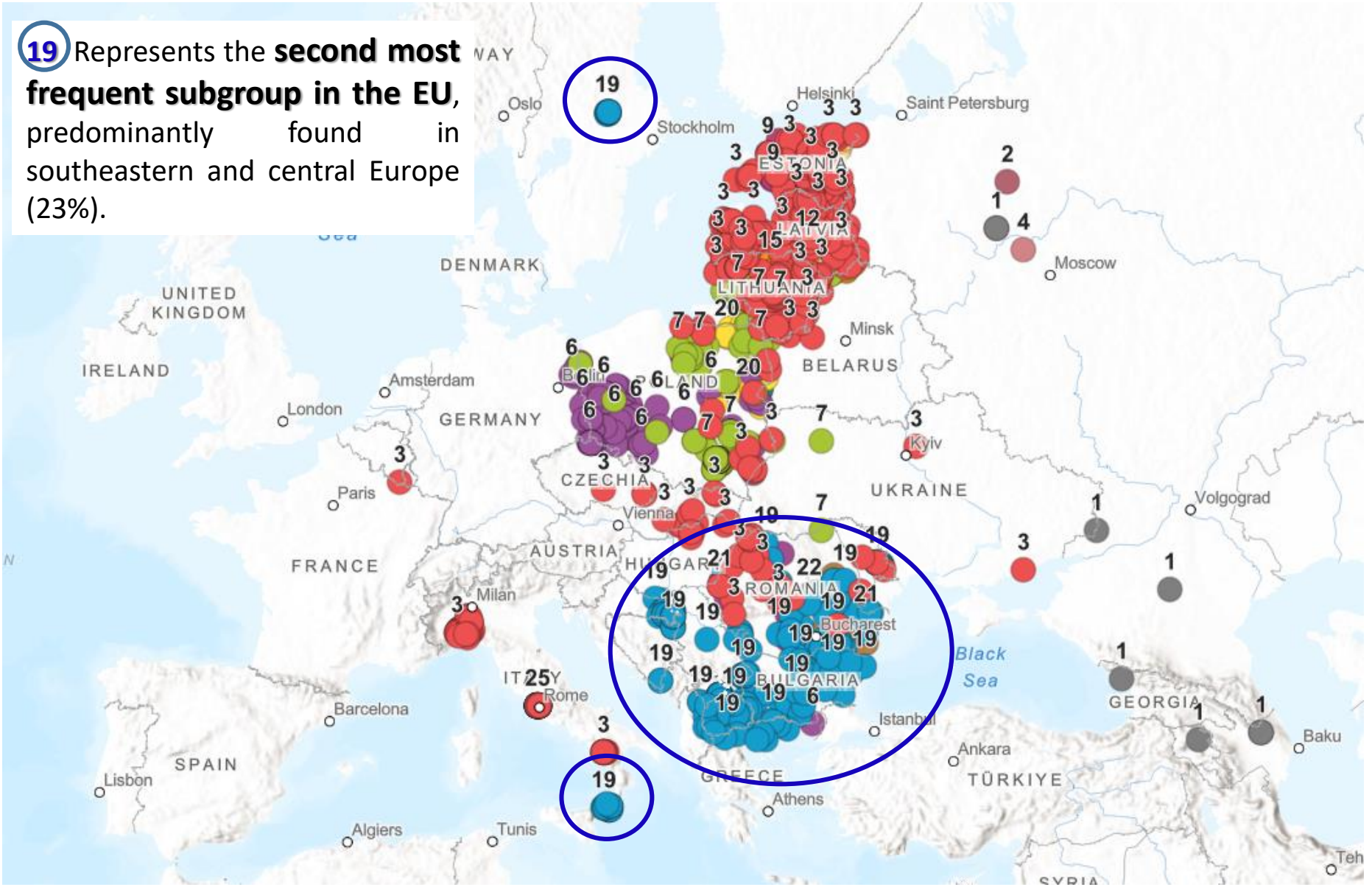
IGR-I → original lineage (Georgia 2007)

IGR-II → dominant lineage circulating in Europe (>95%)

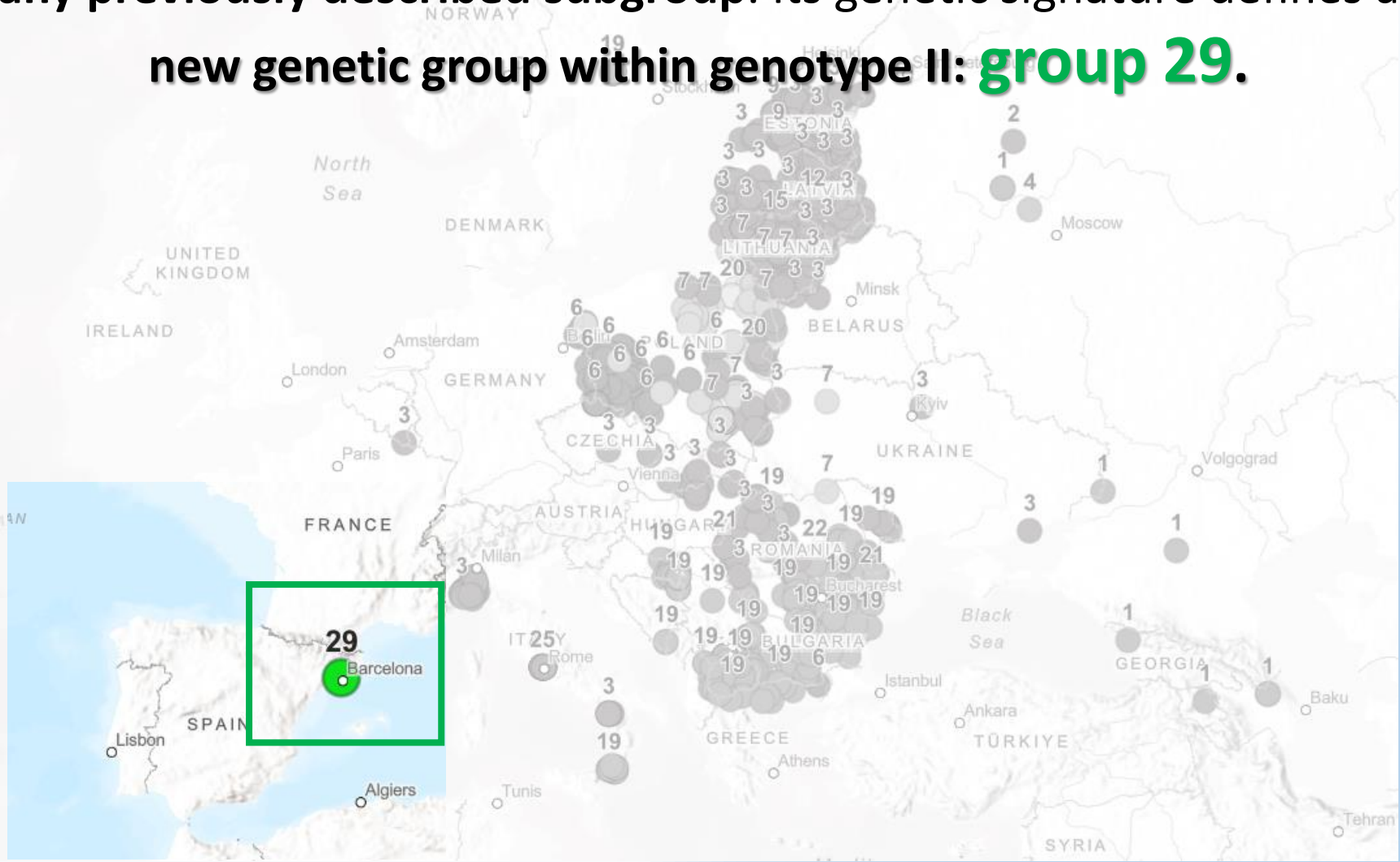

1 Represents the **starting point** associated with the initial outbreak in **Georgia (2007),** which initiated the current pandemic (0.9%).
Oslo
Helsinki
Saint Petersburg
Stockholm
ESTONIA
LATVIA
LITHUANIA
Moscow
DENMARK
UNITED KINGDOM
IRELAND
Minsk
BELARUS
Amsterdam
London
GERMANY
POLAND
Kyiv
Paris
CZECHIA
UKRAINE
Volgograd
Vienna
AUSTRIA
HUNGARY
FRANCE
ROMANIA
Milan
Bucharest
Black Sea
BULGARIA
GEORGIA
Barcelona
Rome
Istanbul
Baku
Ankara
SPAIN
Lisbon
GREECE
TÜRKIYE
Athens
Algiers
Tunis


3 Associated with the first outbreaks detected in the EU in 2014 and represents the predominant subgroup (37%).
Helsinki
Saint Petersburg
Stockholm
ESTONIA
LATVIA
LITHUANIA
Moscow
Minsk
BELARUS
DENMARK
North Sea
UNITED KINGDOM
IRELAND
Amsterdam
London
GERMANY
POLAND
CZECHIA
Vienna
Lviv
UKRAINE
Volgograd
FRANCE
Paris
AUSTRIA
HUNGARY
ROMANIA
Bucharest
Milan
Rome
Barcelona
SPAIN
Lisbon
Black Sea
BULGARIA
GEORGIA
Istanbul
Ankara
TÜRKIYE
Baku
GREECE
Athens
Algiers
Tunis
SYRIA

19 Represents the **second most frequent subgroup in the EU**, predominantly found in southeastern and central Europe (23%).



The virus responsible for the Spanish outbreak **does not belong to any previously described subgroup**. Its genetic signature defines a **new genetic group within genotype II: group 29.**



Why is it classified as genetic group 29?

Variant / Group	CVR	IGR	O174L	K145R	MGF	ECO2
Group 1 - Georgia 2007	● I	● I	● I	● I	● I	● I
Group 29 –Spain 25	● I	● I	● I	● I	● I-V2	● I

● 5/6 loci identical to the Georgia 2007 lineage

● **Novel SNP in MGF505-9R/10R intergenic region** → **MGFI-V2**

RESULT

- Virus closely related to group 1 (original lineage)
- Presence of a unique genetic difference

☞ **Definition of a new genetic group: 29**

→Where does this virus come from?

→ How did it reach this area?

Level 1.
GENOTYPE 2 (G2)
current pandemic
Low resolution

Single gen/24 genotypes described

Level 2.
G2, GROUP 29
Not previously described
Related to Georgia 2007 lineage
6 genes / 29 genetic groups within genotype II

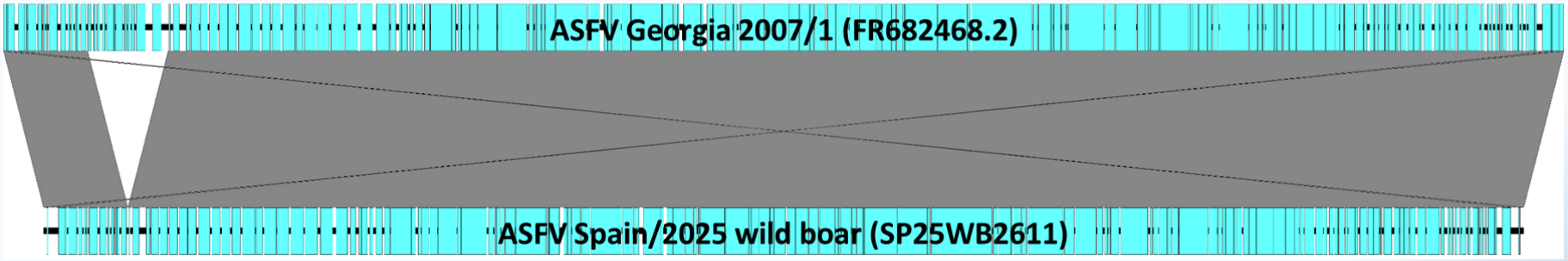
Level 3.
Whole-genome sequencing

Maximum resolution

Answering these questions requires the highest possible genetic resolution.

Level 3 – Whole genome sequencing (2 blood samples case 1 and 2)

9.8 kb deletion (LVR)



- **9.8-kb deletion within the left variable region (LVR)** (positions 10,264–20,087 of the reference genome).
- The deletion removed **21 coding sequences, predominantly members of the MGF110**, together with **MGF360-4L, MGF360-6L, and MGF100-1R**

Whole-genome comparison with the Georgia 2007/1 reference genome

>99.9% nucleotide identity outside the left variable regions (LVRs)

Unique variants identified in the Spanish ASFV genome		
Gene	**Mutation**	**Protein effect**
MGF110-3L	T→C SNP	No amino-acid change
MGF505-1R	GATAC insertion	Frameshift (531 aa → 485 aa)
MGF360-12L	A→G SNP	No amino-acid change
MGF505-10R	C→T SNP	Frameshift
A859L	G→C SNP	Pro406Ala
EP402R (CD2v)	T→C SNP	No amino-acid change
B962L	T→A SNP	No amino-acid change
B438L	C→T SNP	No amino-acid change
B407L	C→T SNP	No amino-acid change
CP2475L (pp220)	A→G SNP	Thr119Ile
CP2475L	G→A SNP	No amino-acid change
NP1450L	T→C SNP	No amino-acid change
D1133L	T→C SNP	No amino-acid change
D117L	G→A SNP	No amino-acid change
E183L (p54)	T→C SNP	No amino-acid change
MGF360-16R	C→T SNP	Arg71Trp

30 nucleotide differences detected (18 SNPs and 12 indels), including **16 coding variants unique to the Spanish virus**

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16 coding variants unique to the Spanish virus

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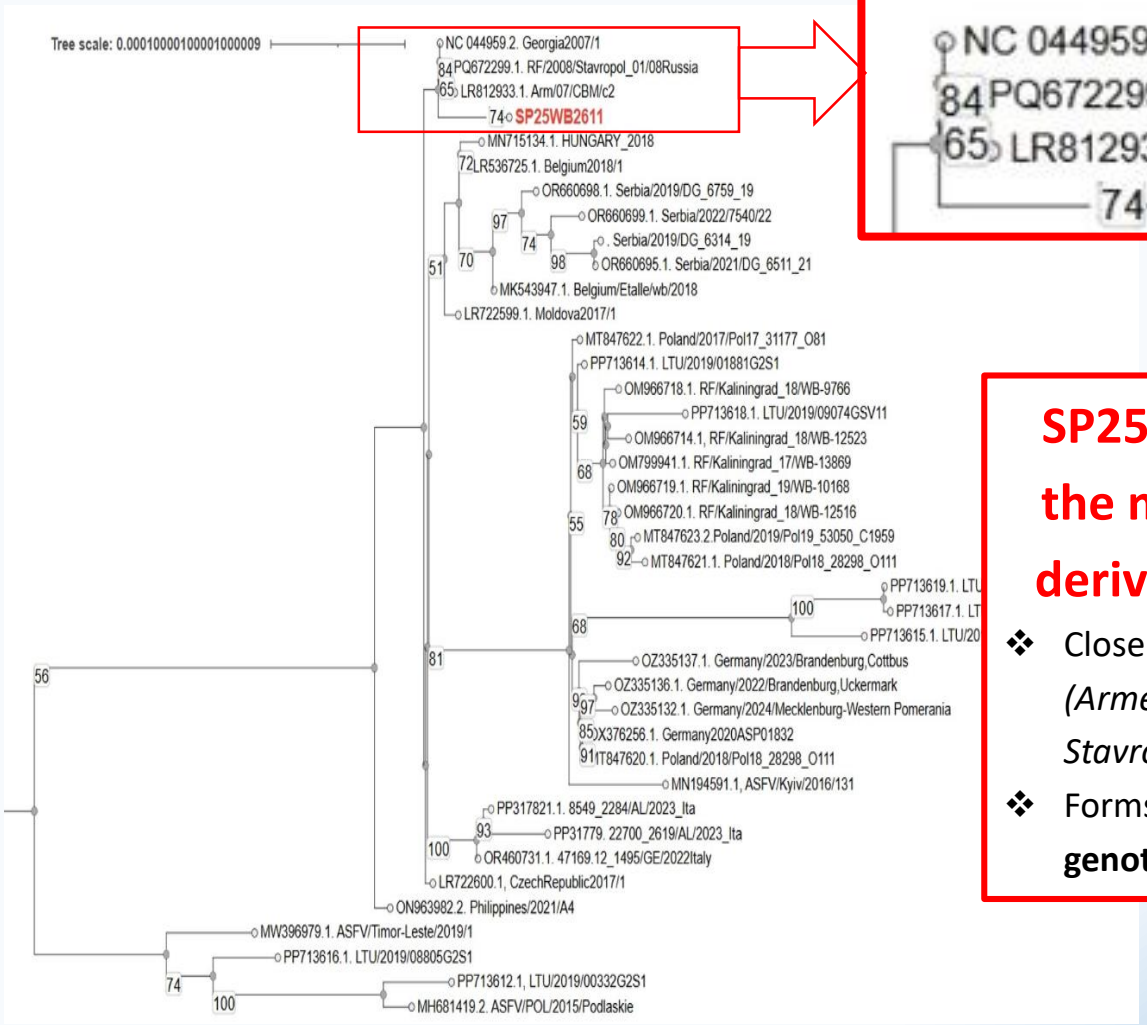
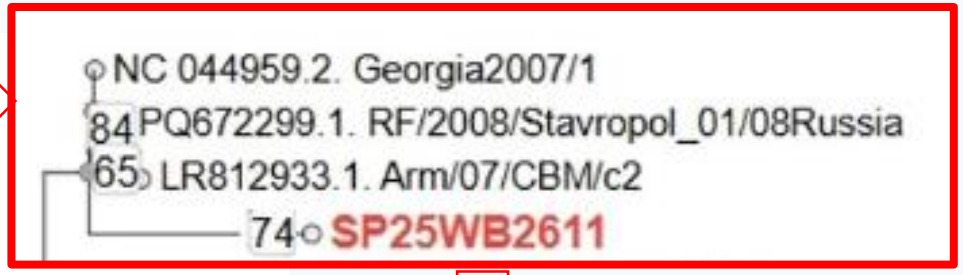
Good genetic markers

Level 3 –Phylogenetic analysis



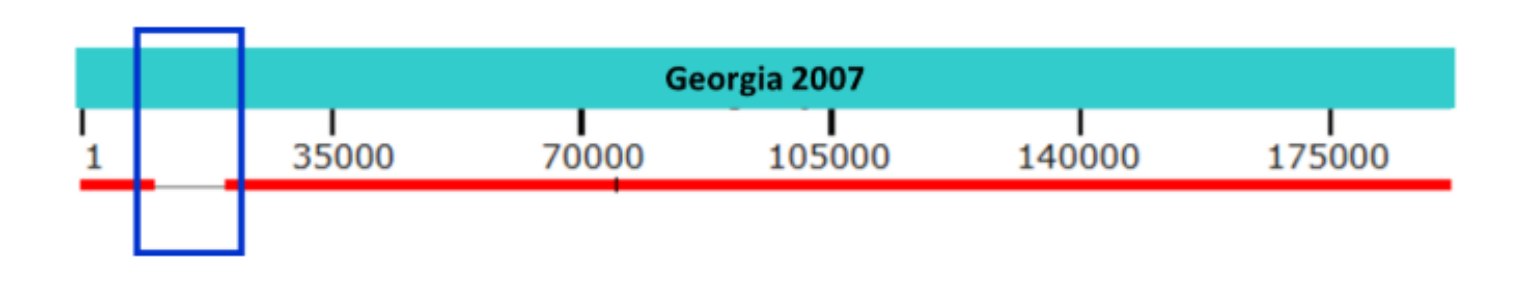


SP25WB2611 clusters within the main genotype II lineage derived from Georgia 2007/1

- Closely related to **early Caucasus viruses** *(Armenia 2007; Russian Federation 2008 – Stavropol)*
- Forms a **distinct branch within the genotype II clade**

Dataset: 40 ASFV genotype II genomes (≥99.94% identity with SP25WB2611)Analysis: Maximum-likelihood phylogeny

Level 3 – Whole genome sequencing/ maximun resolution.

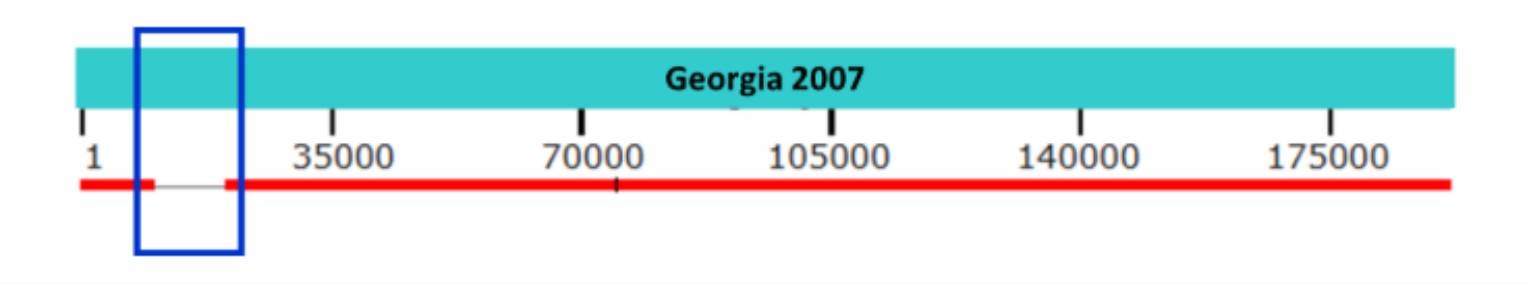


Unique genetic signature of the Spanish virus

- ~9.8-kb deletion in the LVR **affecting MGF genes implied in virulence**
- Distinct branch within the **Georgia-derived genotype II lineage**
- 30 nucleotide differences **(16 unique coding variants very useful as genetic markers)**

Futher ASFVs Spanish cases have been confirmed to have this unique profile (partial sequencing of MGF505R and p54)

Level 3 – Whole genome sequencing/ maximun resolution.



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- ~9.8-kb deletion in the LVR **affecting MGF genes implied in virulence**
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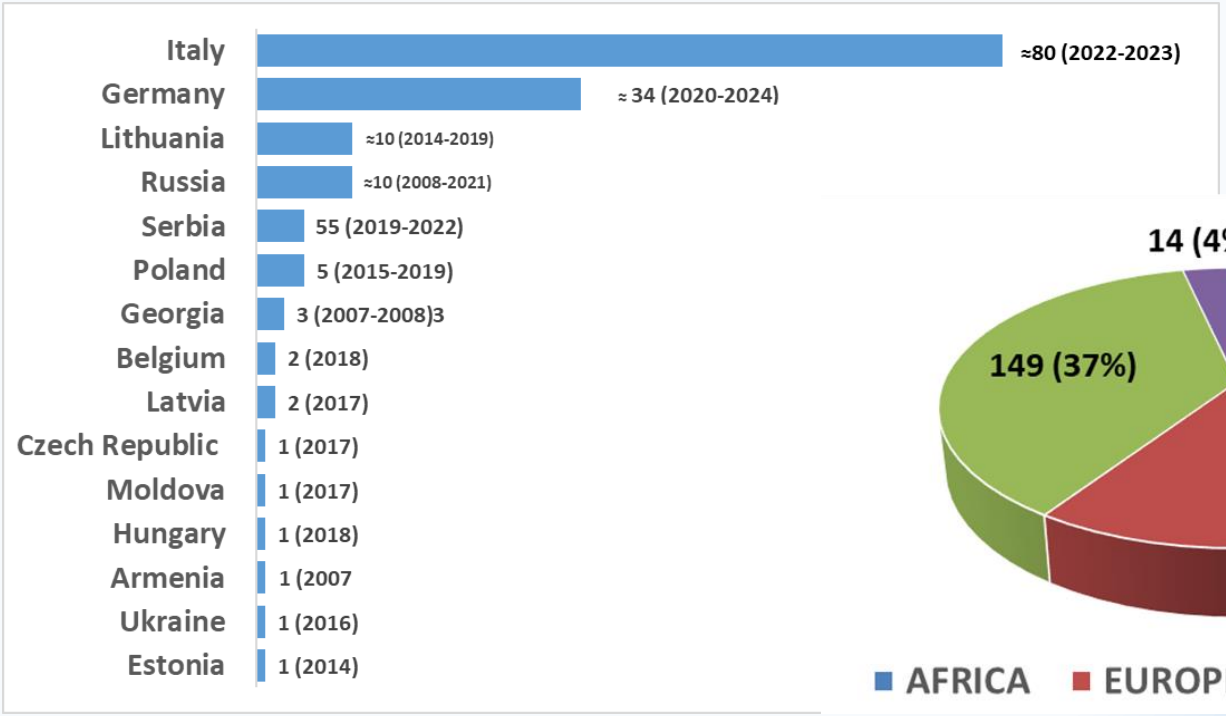
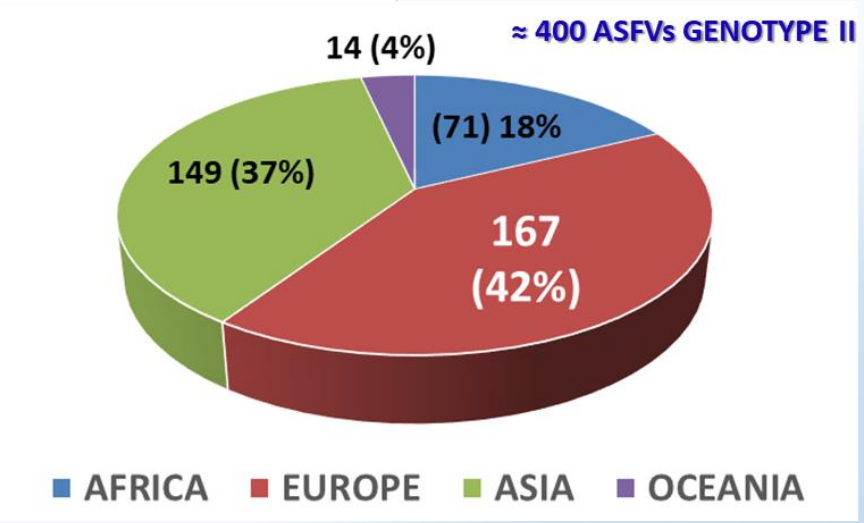
- 30 nucleotide differences **(16 unique coding variants very useful as genetic markers)**

The unique genetic signature not found in other ASFVs public databases and therefore we cannot determine the geographic origin of the oubreak.

Genomic analysis: scope and limitations

Limited number of available complete ASFV genomes from Europe due to technical constraints (large genome, TIR, TRS, polynts)



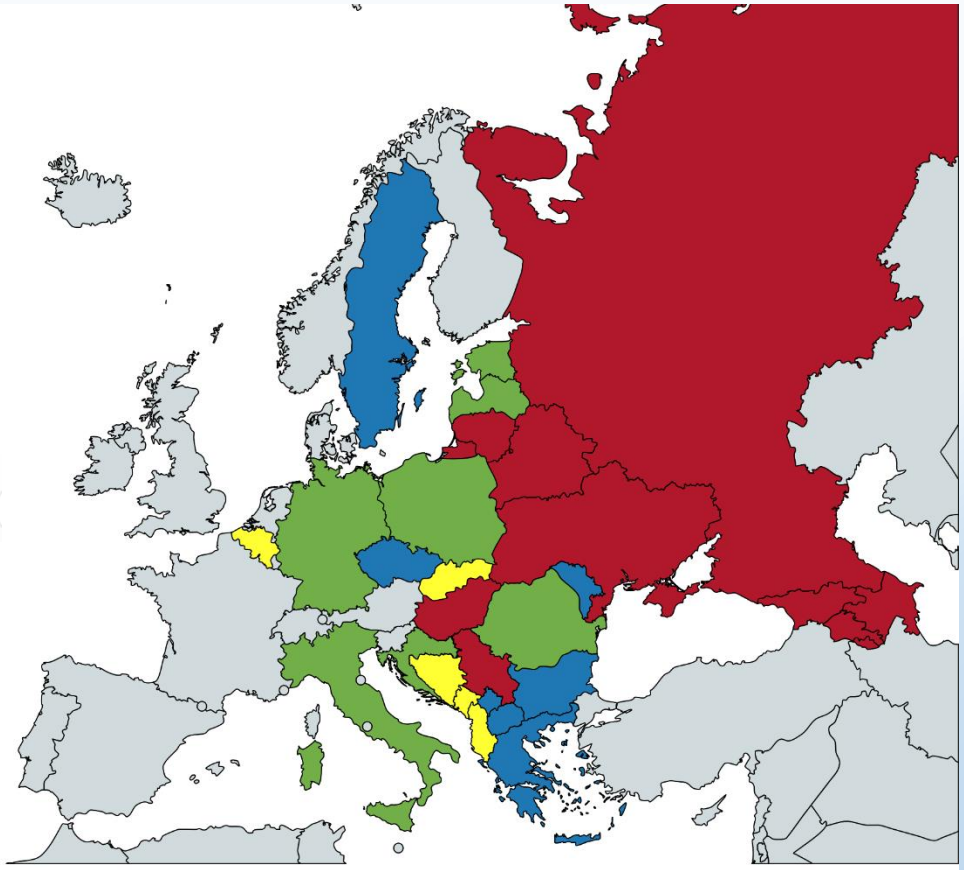


Gaps in complete ASFV genome data prevent precise reconstruction of the outbreak origin.

Genomic analysis: scope and limitations

Availability of ASFV multigene data across Europe (1,257 ASFV sequences)

- **Category 1 – Extensive recent genetic data**
  (≥50 sequences, last data within 3 years)
- **Category 2 – Limited recent genetic data**
  (5–49 sequences, last data within 3 years)
- **Category 3 – Very limited recent genetic data**
  (<5 sequences, last data within 3 years)
- **Category 4 – Genetic data gap**
  (no recent genetic data available)

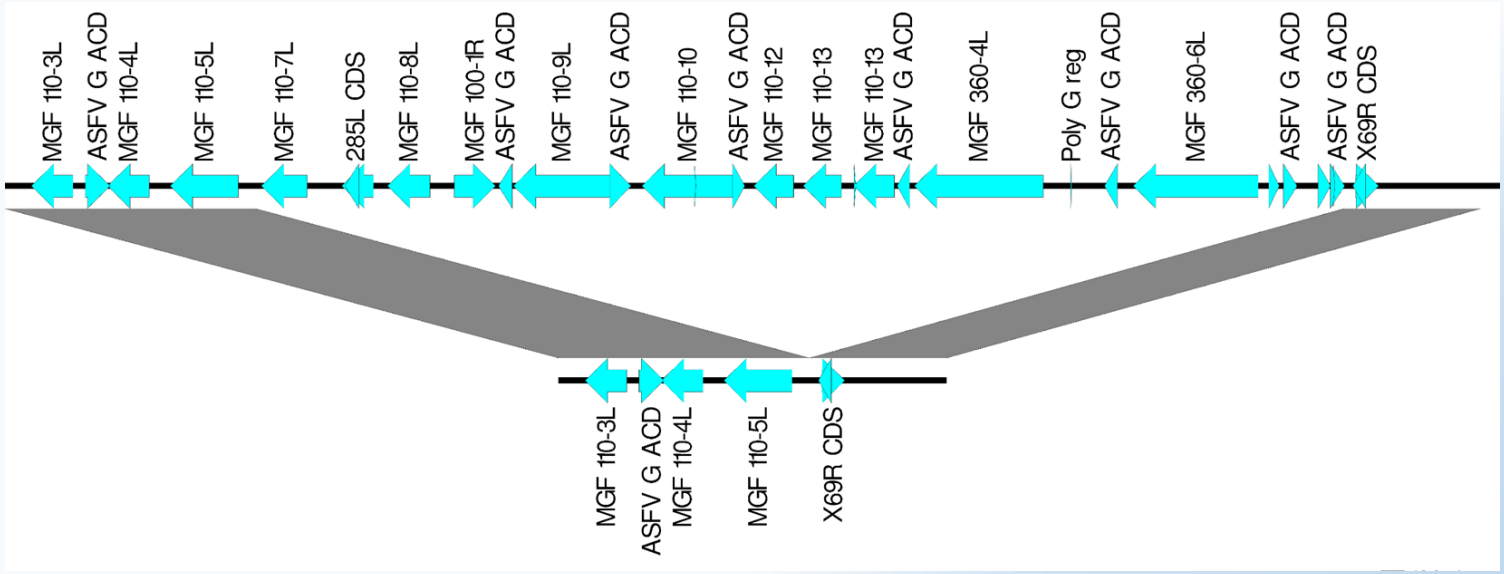


Genetic data gaps persist even with the multigene approach

Key genomic findings

From a genomic perspective, **the origin of the outbreak could not be precisely determined, but:**

- Identification of a **genetically distinct ASFV variant (genetic group 29)**
- **Unique genomic signature**, including loss of genes potentially involved in virulence (MGF110 gene family)



Conclusions

Virus characterization

- A **genetically distinct ASFV genotype II variant (new genetic group 29)** was identified
- **Large genomic deletion affecting 21 genes mainly from the multigene family MGF110 and MGF360 → posibles implications in virulence?**
- **16 unique variants out of the 30 identified not previously described**

Origin of the outbreak

- **Possible scenario: Single human-mediated introduction**, most likely via **contaminated pork products or food waste**
- The **precise origin of the outbreak could not be reconstructed** due to **gaps in available genetic data across Europe and neighboring regions**
- **Singular introduction event** from an undersampled area, or an area with a gap in genetic data.

Implications for genomic surveillance

Strengthening ASFV genomic surveillance is essential

- **Level 2 – Multigene typing** provides a practical tool for large-scale monitoring
- **Level 3 – Whole-genome sequencing** remains essential for high-resolution investigation of selected outbreaks
- The best strategy **should be discussed and supported** since is not feasible to perform whole -genome sequencing in all ASFVs
- **International collaboration and sequence data sharing (EURL ASFV databank)** are critical to improve outbreak tracing.

Improving ASFV genomic data availability is essential to better understand and trace future emergence events.

Muchas gracias por vuestra atención