



Ministero della Salute

ASF situation in Italy

**Directorate General for Animal
Health and veterinary medicinal
products**



Main topics

- Epidemiological situation in Italy
- Proposal of revision of restricted zones
- First results of the phylogenetic study

List of Regions/Provinces with restricted zones in 2024

Genotype I:

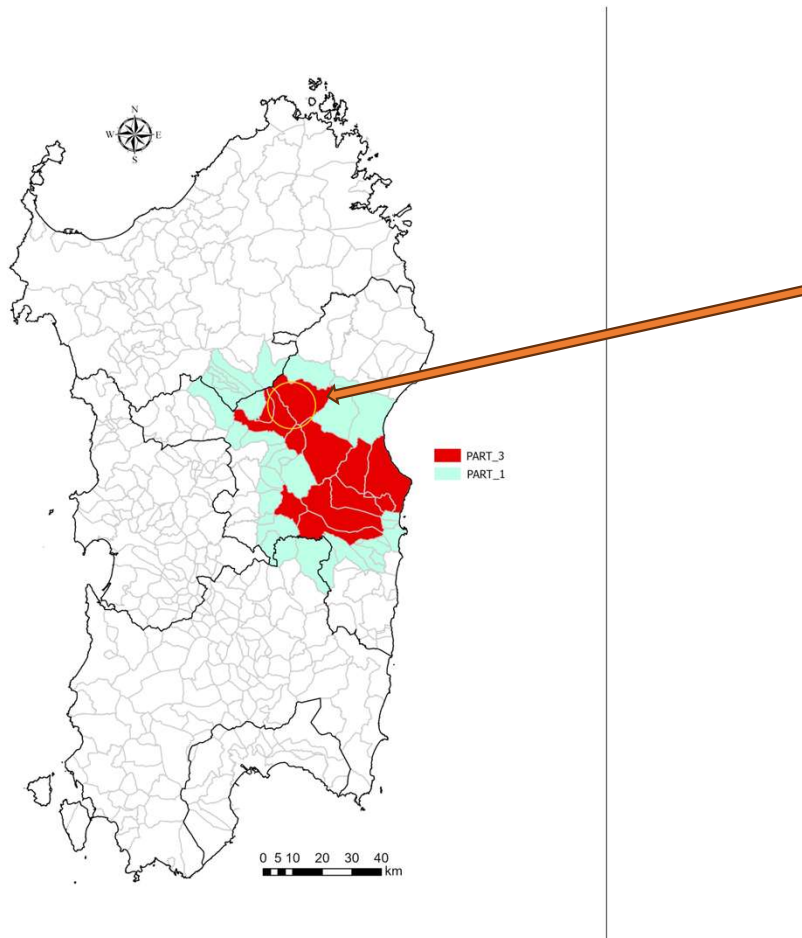
- Sardinia – Nuoro, Sassari and South Sardinia – 1 outbreak in a farm in Nuoro serologically confirmed in January 2023. No detection in wild boar population

Genotype II

- Piedmont – Alessandria, Asti, Cuneo and **Novara**
- Liguria – Genoa, Savona and La Spezia
- Emilia Romagna – Piacenza and Parma
- Lombardy – Pavia, Milan and **Lodi**
- Lazio – Rome
- Campania – Salerno
- Basilicata - Potenza
- Calabria – Reggio Calabria
- **Tuscany – Massa Carrara**

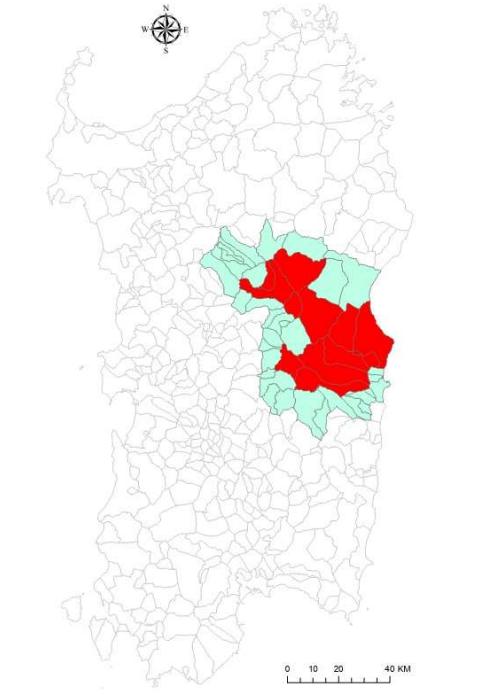


Genotype I – outbreak IT-ASF-2023-00070



- Small closed-cycle breeding farm in Nuoro Municipality (Nuoro Province).
- Seropositive confirmation on 20 January 2023 in a 7 years old sow in a backyard farm with a total of 14 pigs
- Last evidence of genotype I serological confirmation
- Regular surveillance activity on domestic slaughtering

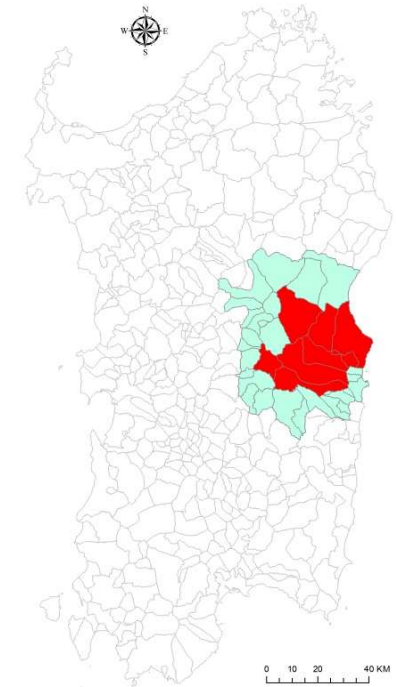
Proposal of lifting of Municipalities in zone I and III



Commission Implementing
Regulation 2024/483

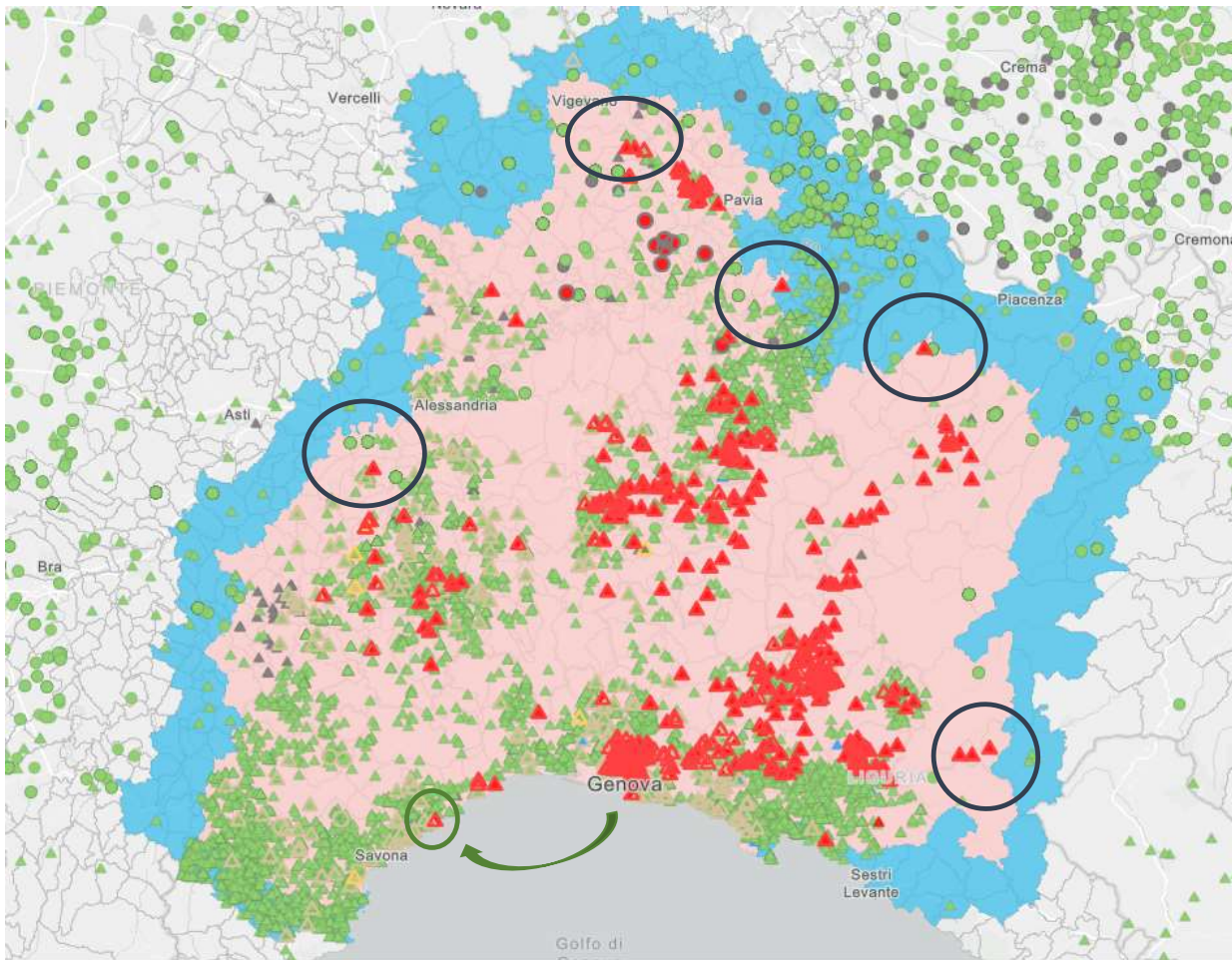
Compliance with conditions listed in the new ASF guidelines at point 6:

- No outbreaks in kept animals and wild boars in the last 12 months
- Active and passive surveillance implemented in domestic and wild animal sectors with favourable results
- compliance with Articles 15, 26 and 41 of Delegated Regulation (EU) 2020/687
- No evidence of presence of *Ornithodoros* ticks



New proposal

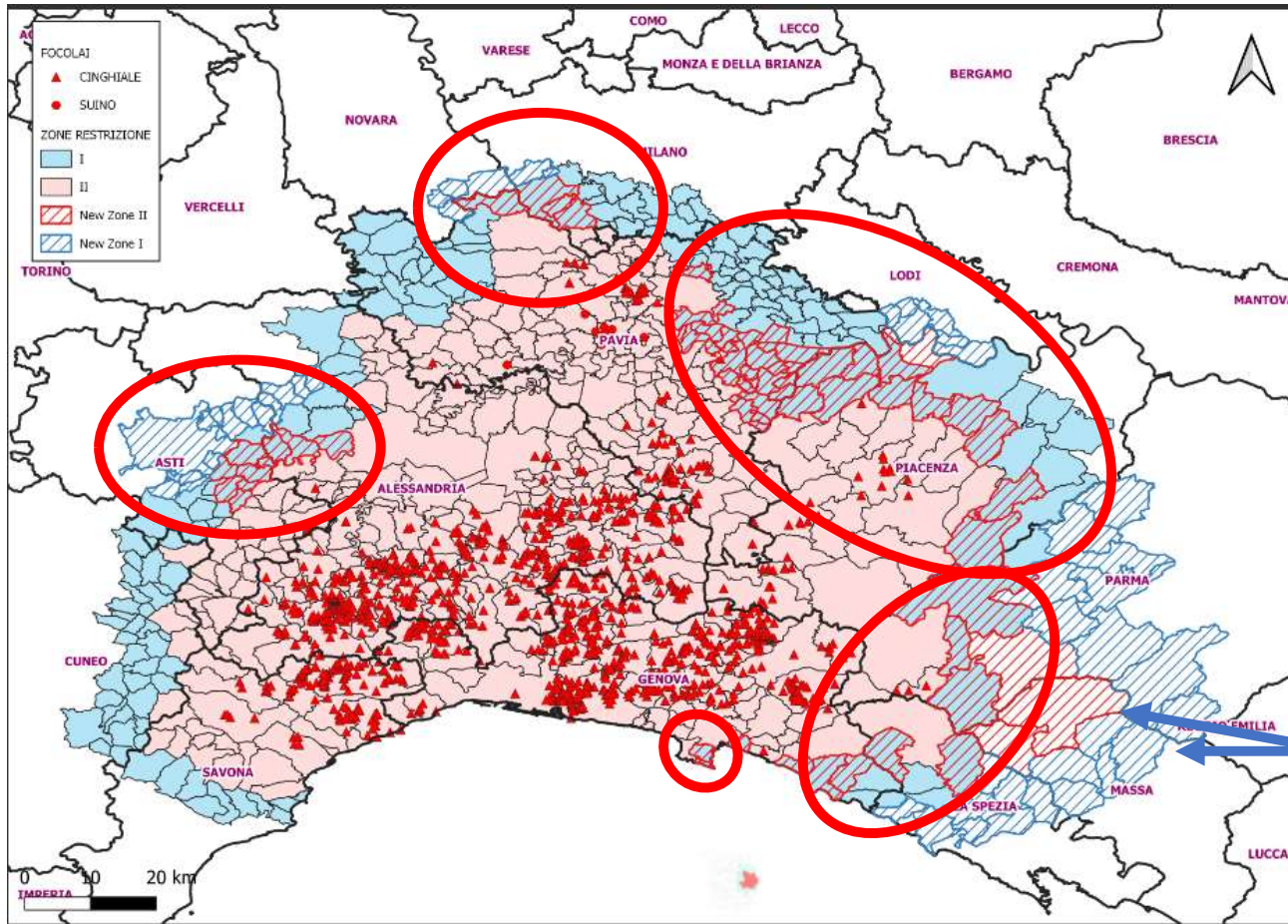
Northern Italy



From the beginning of February several wild boars were tested positive in Piedmont, Liguria, Emilia Romagna and Lombardy regions, close to zone II borders established with Commission Implementing Regulation (EU) 2024/483 of 25 January 2024 amending Annexes I and II to Implementing Regulation (EU) 2023/594. Most of the dead wild boars were young adults and carcasses were fresh.

The case circled in **green**, confirmed on 14/02, was a beached wild boar, most likely brought by sea currents from Genoa area, as in general happens with dead marine animals. In fact, the carcass was swollen with water.

Proposal of amendement of restricted zones in Northern Italy



Following those positive cases in Emilia Romagna, Lombardy, Piedmont and Liguria Regions Italy proposes to enlarge Zones I and II in

- Piacenza and Parma Provinces in Emilia Romagna
- Alessandria, Asti and Novara (new) Provinces in Piedmont
- Genoa and La Spezia Provinces in Liguria
- Milan, Pavia and Lodi (new) Provinces in Lombardy
- New zones II and I in Tuscany, in Massa Carrara Province



CRN per lo Studio delle Malattie
da Pestivirus e da Asfvirus



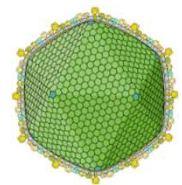
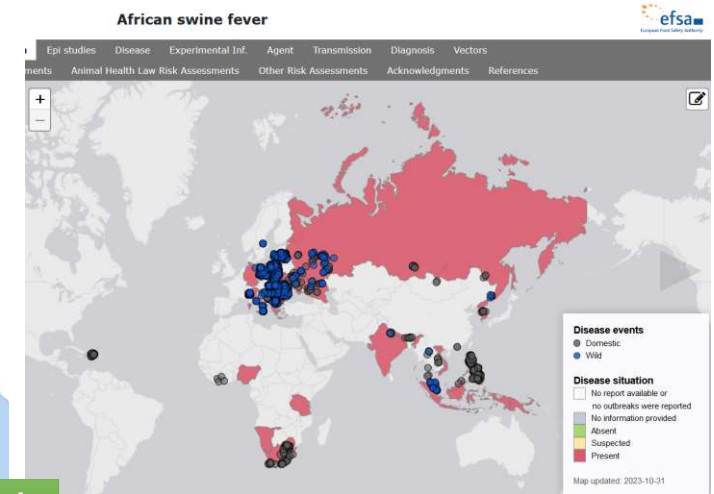
Genetic characterisation of ASF Italian clusters in the 2022-2023 epidemic wave

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Istituto Zooprofilattico Sperimentale Umbria e Marche “Togo Rosati”, Perugia, Italy

Which is the goal of molecular surveillance ?

- ✓ Follow viral spread and assessment of viral origin
- ✓ Follow virus evolution



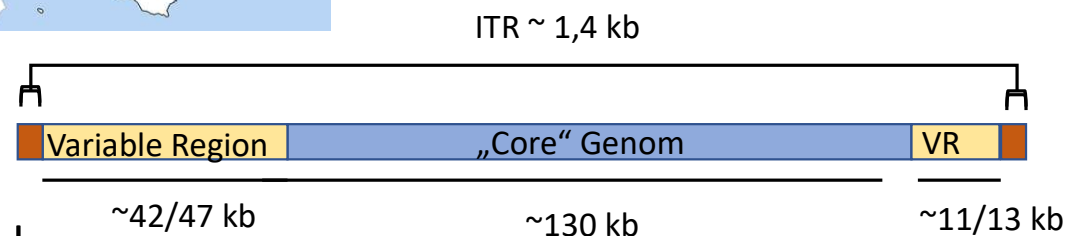
Know your critical spots –

Length: 170.000-193.000 bp

ORFs: 151-167 (ambisense)

Genome ends: ITR, covalently closed

Sequence motifs: repeats, homopolymer regions (>15 C/G)





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ASFV molecular characterization: **our approach**

ASFV genes Sanger sequencing

Essential Genetic Markers

Additional Genetic Markers



1. B646L gene/p72

Genotyping marker

2. B602L gene/CVR
3. IR I73R/I329L/
4. IR MGF-505-9R/10R
5. K145R
6. O174L

Sub-genotyping markers

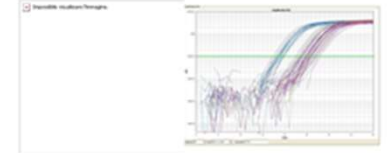
1. IR A179L/A137R
2. MGF-505-5R and MGF-110-7L
3. I267L
4. MGF-360-10L and MGF-505-9R
5. IR C315R/C147L

multi-gene-approach

High-quality *full-genome* sequences

Whole genome sequencing (WGS) – Combine platforms, methods and bioinformatic resources

ASFV Whole Genome Sequencing Workflow



Campioni DNA positivi per ASFV Ct<30



Preparazione librerie con kit Illumina DNA prep (Illumina)



Arricchimento librerie con kit myBaits Custom ASFV (Arbor Biosciences)



NextSeq 2000 (Illumina)



Analisi dati WGS con Genpat



ASFV molecular characterization: **WGS**

CMP_ID	NUM_MAPPED_READS	MIN_VCOV	MAX_VCOV	COV	H_COV	NOTE	PERC_IUPAC	PERC_NS	CONSENSUS_LENGTH
2023.TE.2 0273.1.1	169 353	1	655	114,194	1	mapping on FR682468.2	0	0	190 598

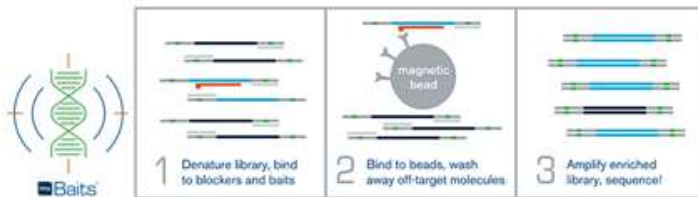


+

CMP_ID	NUM_MAPPED_READS	MIN_VCOV	MAX_VCOV	COV	H_COV	NOTE	PERC_IUPAC	PERC_NS	CONSENSUS_LENGTH
2023.TE.1 9596.1.1	6 217	0	45	4,9228	0,984 946	mapping on FR682468.2	0,005	1,78	190 586



Enrichment of library using myBaits custom ASF



NextSeq 2000
(Illumina)

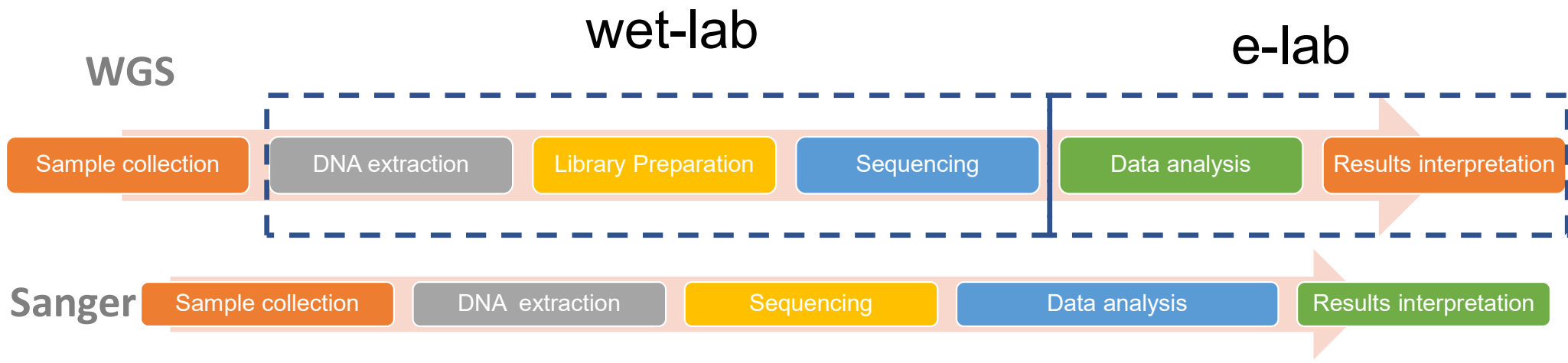


CMP_ID	NUM_MAPPED_READS	MIN_VCOV	MAX_VCOV	COV	H_COV	NOTE	PERC_IUPAC	PERC_NS	CONSENSUS_LENGTH
2023.TE. 21277.1.6	184198	1	1244	142,09	1	mapping on FR682468.2	0	0	190 593





ASFV molecular characterization: **workflow**



- ✓ Spleen/bone marrow – Ct value < 25/30
- ✓ Good sample quality, sufficient viral DNA, high virus/host ratio

January, 5 th, 2022

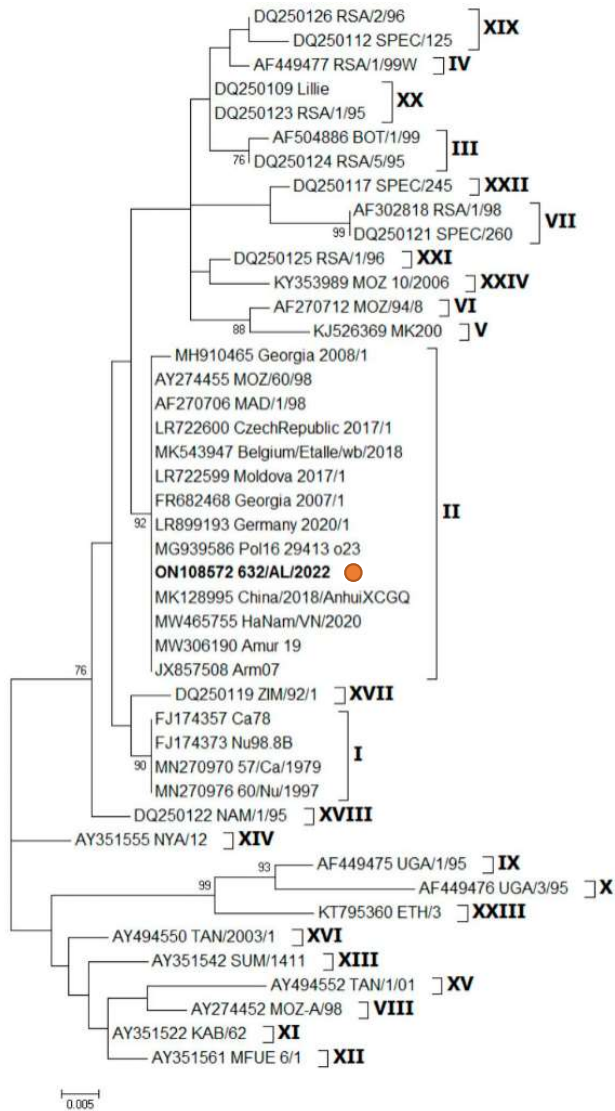
November, 10^o, 2023

111 samples are closed



55 samples are in progress

ASF Italian clusters in the 2022-2023 epidemic wave – phylogenetic analysis



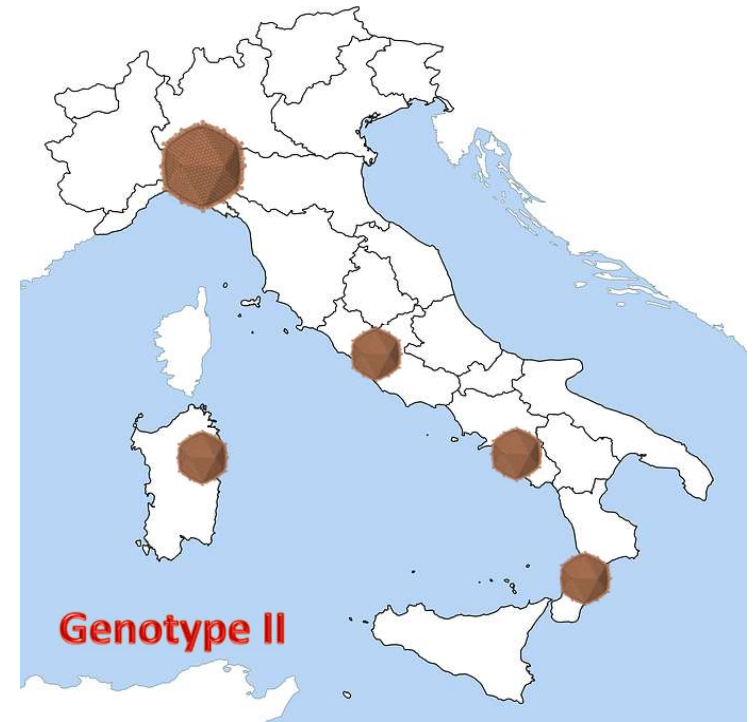
North-West: Piedmont,
Liguria, Lombardia,
Emilia-Romagna

Central: Lazio (ROME)

Sud: Campania

Sud: Calabria

Island: Sardinia





Essential genetic markers (*Gallardo et al., 2023*) – To summarize

Genetic Group	Geographical distribution (year)	CVR	ECO1	O174L	K145R	MGF	ECO2
3	North-East (2022)	I	II	I	I	I	I
19	Lazio (2022)	I	II	I	I	I	II
New ? (25)	Lazio (2023)	I	II	I	I	VIII + new	II
3	North East, Campania, Sardinia (2023)	I	II	I	I	I	I
19	Calabria (2023)	I	II	I	I	I	II

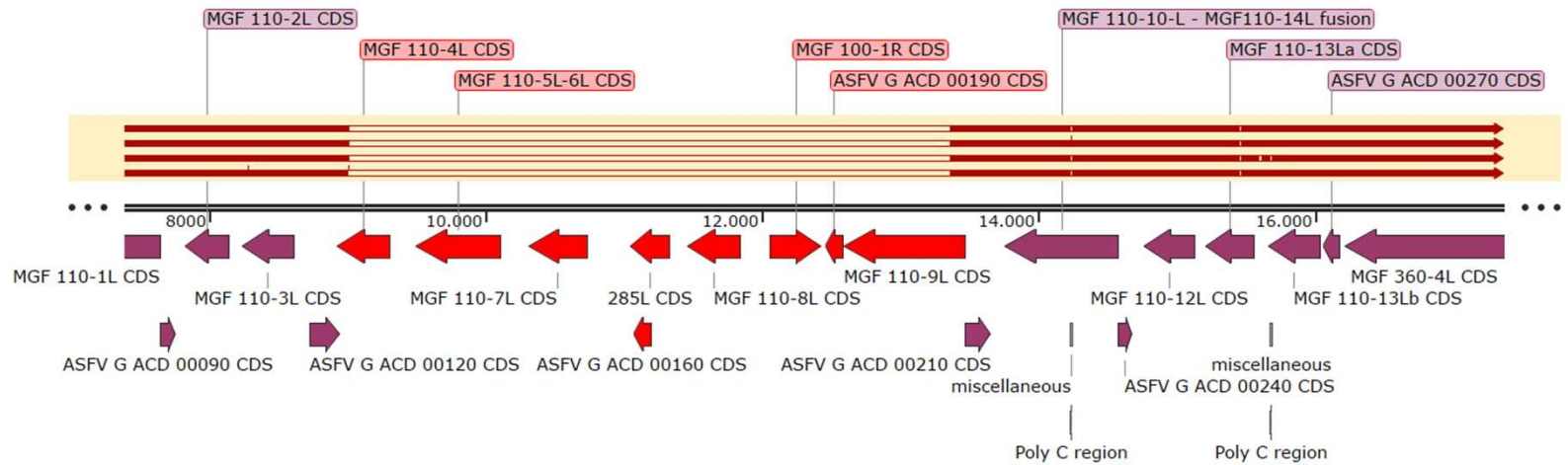


A focus into the 2022-203 ASF Italian outbreaks – WGS

Isolates	Date	5'Del *	length (nt)	genes	3'Del *	length (nt)	genes
8549_2284/AL/2023	11/01/2023	9020-13358	4338*	MGF 110-4L , MGF 110-5L-6L , MGF 110-7L , 285L , ASFV G ACD 00160, MGF 110-8L, MGF 100-1R, ASFV G ACD 00190, MGF 110-9L	----	---	---
22700_2598/AL/2023	14/01/2023	9019-13358	4339*		----	---	---
22700_2645/AL/2023	24/02/2023	9016-13358	4342*		----	---	---



North-West cluster
2023



Georgia 2007 FR682468

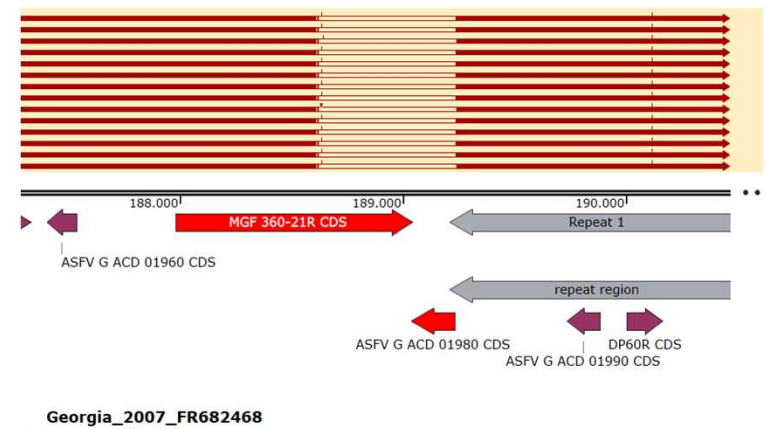
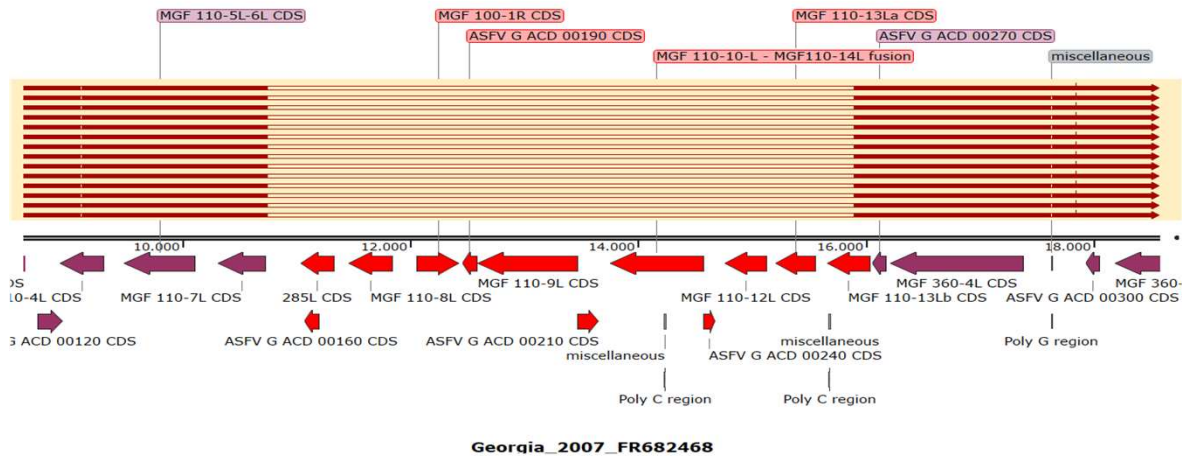


A focus into the 2022-203 ASF Italian outbreaks – WGS



Calabria 2023

Isolates	Date	5'Del *	length (nt)	genes	3'Del *	length (nt)	genes
21896.3_2307/ RC/2023	03- 29/05/2023	10755- 15891	5136	285L , ASFV G ACD 00160, MGF 110-8L, MGF 100-1R, ASFV G ACD 00190, MGF 110-9L, ASFV G ACD 00210, MGF 110-10-L - MGF110-14L fusion, ASFV G ACD 00240, MGF 110-12L, MGF 110-13La, MGF 110-13Lb	188636- 189227	591	MGF 360-21R, ASFV G ACD 01980





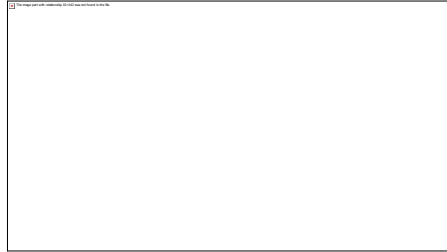
Discussion and consideration

- ✓ Sample with a good quantity and quality is essential
- ✓ We have the best results combine platforms, methods and bioinformatic approaches
- ✓ There is a bias on availability of ASF genotype II sequences in GenBank: partial and *full-length* sequences (and not completely representative of some affected territories)
- ✓ this study will make available many partial sequences (more than 500) and complete sequences (more than 111). These dataset gives an image of different italian infected clusters over the time.



Discussion and consideration

- ✓ On the basis of the actual sequence dataset (*the dataset will be further expanded*), the use of the **multi-gene approach** was useful in identifying the presence of some Single Nucleotide Polymorphisms (SNPs), **two different genetic groups** associated with different clusters and a putative new variant/genetic group related to a specific area
- ✓ The use of additional essential markers could give an update. SNPs and deletion of complete gene were identified
- ✓ The **full-genome approach** allowed the identification of 5' and 3' **deleted whole genome sequences**, and give us a more extensive information about the virus evolution.
- ✓ At the moment it is not yet possible to identify the origin of the infection, but it is possible to hypothesize **two independent introductions in Italy** during 2022 and a **molecular evolution of ASF virus over the time** in different scenarios.



Thanks for your kind attention