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## A Naturally Occurring Microhomology-Mediated Deletion of Three Genes in African Swine Fever Virus Isolated from Two Sardinian Wild Boars

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### Introduction

African swine fever virus (ASFV) is the etiological agent of a lethal disease of domestic pigs and wild boars, which threatens the pig industry worldwide(1). The disease has been endemic for more than 40 years in Sardinia (Italy), but an intense campaign pushed it close to eradication; virus circulation was last detected in 2019 (2).

### Methods

Primary swine macrophages were infected with ASFV strains 7212WB/19 and 7303WB/19 (5). Viral DNA was extracted from cell culture supernatant using a QIAmp Ultrasens Virus Kit (Qiagen) and used for molecular analysis and for Next Generation Sequencing (NGS). Whole-genome sequencing was carried out using Illumina platform. Genome data processing was performed using an in-house bioinformatic pipeline. To investigate the evolutionary relationship of the 7303WB/19 and 7212WB/19 isolates, a phylogenetic analysis was performed using an alignment consisting of 75 WGSs (3) generated from other ASFV isolates from Sardinia (1978 and 2019), Europe and Africa.

### Results

The ASF viruses analyzed in this study were isolated in January 2019 from two wild boars hunted in two distinct hunting management units. By reference aligning the NGS reads, a significant drop in read depth was observed between 11 kb and 17 kb from the 5' end of the genome. The lack of coverage in this region pointed to the presence of a deletion in the genomic sequence of both isolates. The complete deletion was about 4000 base pairs and involved the coding regions of the genes MGF360-6L, X69R and MGF300-1L.

The flanking regions of the deletion were characterized by a sequence of 48 bases, that is found in all Sardinia ASFV isolates. Both regions involved in this microhomology appear to be conserved among all published Sardinian isolates sampled until 2018, but not among ASFV genotype I isolates outside Sardinia. The presence of the microhomology in correspondence to the flanking regions of the deletion suggests that this molecular signature arose as a result of mechanisms related to non-allelic homologous recombination (NAHR) or microhomology-mediated end-joining (MMEJ) (4,5). Phylogenetic analysis showed that both ASFV isolates are assigned within the clade grouping all Sardinian isolates and, therefore, evolving from the Sardinian epidemic (figure 1). Their most recent common ancestor gave rise to a minor clade containing a few recent Sardinian viruses from 2004 to 2015 and is estimated to have been circulating during late 2012.

### Conclusions:

This study describes for the first time two Sardinian ASFV isolates with a sustain deletion in their genome (4342 bases near the 5' end). Genomic analyses suggest that this deletion was most likely a result of a non-allelic homologous recombination driven by a microhomology. Our results raise questions on the functions of the deleted genes and, whether this ASFV variant has biological implications for ASFV persistence in Sardinia or whether its appearance is linked to the eradication campaign carried out in recent years.