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Genomic analysis of Sardinian 26544/OG10 isolate of African swine fever virus

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ABSTRACT

Comparative genomic analysis aims to underscore genetic assortment diversification in distinct viral isolates, to identify deletions and to carry out evolutionary studies. We sequenced the first complete genome of an ASFV p72 genotype I strain isolated from domestic pigs in Sardinia (Italy) using Next-Generation Sequence (NGS) technology. The genome is 182,906 bp long, contains 164 ORFs and has a 99.20% nucleotide identity to the L60 strain. Comparison analysis against the 16 ASFV genomes available in the database showed that 136 ORFs are present in nine ASFV isolates annotated to date. The most divergent ORFs codify for uncharacterized proteins such as X69R and DP96R, which have 51.3% and 70.4% nucleotide identity to the other isolates. A comparison between the Sardinian isolate and the avirulent isolates OURT 88/3, NHV, BA71V was also carried out. Major variations were found within the multigene families (MGFs) located in the left and right genome regions.

1. Introduction

African swine fever (ASF) is an extremely contagious viral disease affecting domestic pigs and wild boars, caused by a large cytoplasmic DNA virus, the African swine fever virus (ASFV), the only member of the *Asfarviridae* family. The disease is characterized by haemorrhagic lesions of the skin and other organs and can determine hyperacute, subacute, chronic and unapparent forms (EFSA AHAW Panel, 2014). Endemic among pigs and wild boar populations in Sub Saharan Africa and in Sardinia, Italy (Mur et al., 2014a), ASFV entails severe repercussions for the entire swine farming industry. Currently, there is no therapy or vaccine available against the disease, the only biosecurity measures being primary prevention and proper management of outbreaks. The virus was introduced in Portugal from western Africa in 1957 and in 1960. During the second introduction, ASFV spreads in the whole Iberian Peninsula and from this area to both American and European countries, Sardinia included, where it arrived in 1978. The disease has since been successfully eradicated in all these territories except Sardinia (Mur et al., 2014a,b) where it is still endemic in spite of control and eradication plans adopted since 1982. The virus entered Georgia in 2007 coming from East Africa (Sánchez-Vizcaíno et al., 2015) and spread rapidly throughout the rest of the Caucasus and then in the Russian Federation. Since 2014, outbreaks of African swine fever were reported in the southern Baltic countries, Estonia, Latvia, Lithuania and Poland.

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Recent genotyping studies (Sanna et al., 2016; Giammarioli et al., 2011) showed that the Sardinian strains can be distinguished in two subgroups temporally related on the base of the analysis of two genes, the EP402R gene, encoding for the CD2v protein and the B602L gene. All the viruses isolated after 1990, with some exceptions, showed deletions respect to those isolated before 1990. Then during the spreading in Sardinia, since 1990 the virus has undergone genomic variations in at least two genome regions with respect to its ancestor. Therefore it is of great interest to perform a comparison between the viruses which are now circulating in Sardinia and the international related strains. The recent development of next-generation sequencing technologies allows whole-genome virus sequencing at a relatively low cost. This knowledge along with the comparison between virulent and avirulent strains might bring new insight into virus evolution but also in host-virus interaction and pathogenicity. In this study, we present the first complete genome sequence of an ASFV p72 genotype I isolate from domestic pigs in Sardinia and we perform a comparative analysis with all the complete genome sequences of ASFV available in GenBank.

2. Material and methods

The primary virulent, hemoadsorbing, virus 26544/OG10 was isolated in 2010 in Sardinia (Italy) from pig tissue samples (spleen) on porcine peripheral blood mononuclear cell (PBMC) as previously described (Malmquist and Hay, 1960). This virus was propagated for three times and then purified (Zhang et al., 2006). Cell supernatant containing the virus was centrifuged at 118,000*g* for 1 h at 4 °C. The virus was purified as described (Chapman et al., 2011), incubated with DNAse and RNAse to digest contaminating cellular DNA and RNA and centrifuged through at 20% sucrose RSB (Resuspension Buffer) cushion at 62,000*g* for 95 min at 4 °C. Viral genomic DNA was extracted through phenol and precipitated using ethanol.

The next generation sequencing was performed at the Porto Conte Ricerche Srl (Alghero, Italy) using the Illumina HiScanSQ platform. Briefly, an aliquot of DNA (5 μL at 0.2 ng/μL) was processed with the Nextera XT kit and libraries were sequenced with 2 × 93 bp paired-end reads. High-quality reads were mapped against the pig genome (Sscrofa10.2, GenBank assembly accession: GCA_000003025.4) eliminating over 95% of host DNA contamination. About 40,000 viral reads were then assembled using Velvet de novo assembler 1.2.10 (Zerbino and Birney, 2008) into 98 contigs bigger than 100 bp. The contigs were ordered using the other ASFV genome references available on NCBI and the remaining gaps were closed via PCR and Sanger sequencing. In addition, the repetitive regions were confirmed via Sanger sequencing to avoid misassembling. The genome sequence of 26544/OG10 was annotated and global aligned against sixteen ASFV complete genomes available on NCBI, nine of which fully annotated (using the Needleman-Wunsch algorithm implemented inside EMBOSS Stretcher). Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013). The phylogeny of single proteins was inferred by using the Maximum Likelihood method based on the JTT + G matrix-based model (Jones et al., 1992). A bootstrap analysis based on 1000 replicates was performed to assess the robustness of individual clades. The whole genome sequences of 16 isolates were aligned against 26544/OG10 with CSI Phylogeny 1.1 webserver (Kaas et al., 2014) in order to find SNPs in core regions and the phylogenetic tree was built using Neighbor-Joining. DAVID v6.7 was used to gain biological insight from the gene lists, to identify enriched GO terms, and to find functionally related gene groups (Huang da et al., 2009a,b). This analysis was also performed for the gene list of isolates belonging to genotype I and genotype II. From the functional clustering analysis, we selected the top four clusters for each processed gene list based on the enrichment score, where a higher enrichment score indicates closer agreement among terms. The graphic representation of genomes and proteins of alignments were made using Geneious 4.8.5.

Table 1
Genomic sequences of the ASFV strains used in this study. The table indicates the accession number, the number of CDS (when annotated), the size of the genome, the country and the year of isolation, the host, and the reference (if published). The last column indicates the global alignment percentage identity between 26544/OG10 and each strain.

Strain	Accession	CDS	Length	Country	Host	Year	Reference	Pairwise % identity
26544/OG10	KM102979	164	182,906	Italy	*Sus scrofa*	2010	This study	–
BA71V	U18466	152	170,101	Spain	Tissue culture adapted	1971	Yáñez et al. (1995)	99.2%
Benin 97/1	AM712239	156	182,284	Benin	*Sus scrofa*	1997	Chapman et al. (2008)	99.2%
E75	FN557520	163	181,187	Spain	*Sus scrofa*	1975	De Villiers et al. (2010)	98.6%
Georgia 2007/1	FR682468	188	189,344	Georgia	*Sus scrofa*	2007	Chapman et al. (2011)	92.1%
Ken05/Tk1	KM111294	168	191,058	Kenya	*Sus scrofa*	2005	Bishop et al. (2015)	83.0%
Ken06.Bus	KM111295	161	184,368	Kenya	*Sus scrofa*	2006	Bishop et al. (2015)	84.0%
Kenya 1950	AY261360	–	193,886	Kenya	*Sus scrofa*	1950	De Villiers et al. (2010)	82.3%
L60	KM262844	163	182,362	Portugal	*Sus scrofa*	1960	Portugal et al. (2015)	99.2%
Malawi Lil-20/1 (1983)	AY261361	–	187,612	Malawi	*Ornithodoros moubata*	1983	Haresnape and Wilkinson (1989)	85.2%
Mkuzi 1979	AY261362	–	192,714	Zululand	*Ornithodoros* sp.	1979	Unpub.	91.8%
NHV	KM262845	158	172,051	Portugal	*Sus scrofa*	1968	Portugal et al. (2015)	91.0%
OURT 88/3	AM712240	157	171,719	Portugal	*Ornithodoros erraticus*	1988	Chapman et al. (2008)	91.0%
Pretorisuskop/96/4	AY261363	–	190,324	South Africa	*Ornithodoros porcinus porcinus*	1996	Zsak et al. (2005)	90.7%
Tengani 62	AY261364	–	185,689	Malawi	*Sus scrofa*	1962	Pan (1992)	91.5%
Warmbaths	AY261365	–	190,773	South Africa	*Ornithodoros* sp.	1987	Unpub.	90.7%
Warthog	AY261366	–	186,528	Namibia	*Phacochoerus africanus*	1980	Unpub.	90.7%

3. Results

3.1. Sequencing of the Sardinian isolate 26544/OG10 genome and comparison to other ASFV isolates

We have determined the first complete genome sequence from a p72 genotype I isolated in Sardinia (Italy) in 2010. Genotype I pertinence was confirmed through high quality BLAST, using the existing gene bank sequences of the same genotype (Giammarioli et al., 2011). The complete 26544/OG10 genome sequence was submitted to GenBank/EMBL/DDBJ with accession number KM102979. The genome is 182,906 bp long with an average 38.6% GC content and contains 164 ORFs. The global alignment between the Sardinian isolate genotype I and the other genotypes showed the maximum percentage identity with L60, BA71V and Benin 97/1 (99.2%) and the minimum with Kenya 1950 (82.3%) (Table 1). A visual overview of the similarities between each of the 17 aligned ASFV genome sequences is shown in Fig. 1. Of the 164 ORF, 136 are present in all of the nine ASFV isolates sequenced and annotated to date. 106 ORFs showed amino acid identity exceeding 90% in all of the 16 isolates sequenced. The 164 ORFs in the 26544/OG10 genome fall into two categories, conserved and hypervariable.

3.2. Conserved ORFs

The conserved ORFs encode for structural proteins, transcription and processing factors, enzymes and other proteins involved in nucleotide metabolism, DNA repair and viral replication. Several ORFs were classified as membrane and transmembrane proteins, 18 of which are members of the multigene family (MGF) whereas the remaining 43 are of unknown function. The zinc finger protein B385R, involved in viral transcription regulation, shows a 97.9% amino acid identity in all isolates. The viral protein involved in host-virus interaction, H339R, shows a 97.8% amino acid identity compared to the other 16 isolates analyzed. The O61R p12, involved in virion attachment to host cell, shows a 97.4% identity compared to the other isolates. The most conserved proteins furthermore include several uncharacterized proteins such as E184L (more than 98% similar in all isolates), H124R, M1249L, K145R and G1340L (97.5% identity in the last three). The apoptosis regulating protein Bcl-2 A179L has a 97.7% amino acid identity in all isolates. Most of the conserved proteins are enzymes, including those with catalytic activity and helicases (NP868R, B119L, and Q706L).

3.3. Non-conserved, hypervariable ORFs

The most divergent proteins in the isolate 26544/OG10 include uncharacterized proteins such as X69R and DP96R, which have 51.3% and 70.4% nucleotide identity, respectively, compared to the other isolates. Protein DP96R consists of 127 amino acid residues in Kenyan isolate Ken05/Tk1 whereas only 97 amino acids are found in the other isolates. DP60R, found in isolates

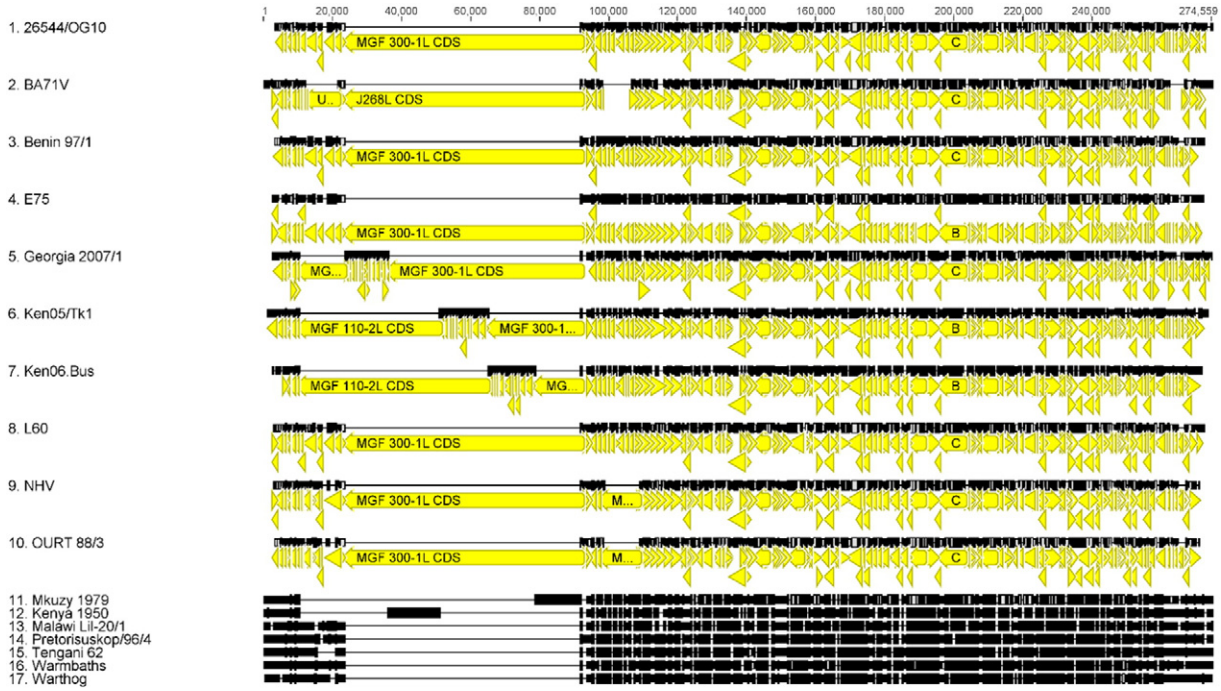

Fig. 1. Genome alignment of the newly determined Sardinian 26544/OG10 isolate to other ASFV isolates. The sequences were aligned using progressive Mauve algorithm inside Geneious.

26544/OG10, L60 and BA71V is not annotated in Benin 97/1. This gene is absent in isolates NHV and OURT 88/3. A protein with immuno-modulatory functions, EP153R, is a type II transmembrane protein containing a C-type lectin domain and similar to NK cell receptors such as CD69. This ASFV encoded protein has been shown to inhibit up-regulation of MHC Class I expression on the cell surface; it also inhibits apoptosis (Hurtado et al., 2004). The transmembrane EP153R in isolate 26544/OG10 is larger than the same protein in OURT 88/3 and in NHV, but shorter in the other isolates Georgia 2007/1, Ken05/Tk1 and Ken06.Bus (Fig. 2A). The CD2-like protein EP402R is hypervariable in the different isolates studied. This protein is directly involved in the haemadsorption of erythrocytes around infected macrophages, its deletion abrogated this phenomenon (Dixon et al., 2004). It is composed of 428 identical amino acids in isolates 26544/OG10, Benin 97/1 and BA71V. It is slightly smaller in isolate E75 (420 amino acids), in OURT/88 and NHV (330 aa) and in L60, which lacks 32 aa in the 175-to-206 region (Fig. 2B). The C-terminal region of the protein contains a tandem repeat sequence of six amino acids ("PPPKPC", repeated seven times) and three variants ("SPPKPC", "RPPKPC" and "PPSKPC") repeated once. Proteins belonging to strains Georgia 2007/1, Ken05/Tk1 and Ken06.Bus do not show a region ranging from residues 373 to 415 and containing one (plus two variants), four and three series of sequence "PPPKPC" tandem repeats, respectively (Fig. 3A). The B602L protein plays a key role in the icosahedral capsid assembly (Dixon et al., 2013). The percentage identity with other isolates equals 88%. This protein contains tandem repetitive amino acids sequences ("CASTC", "DTNVD") which differ in number between the different strains and which have been used for ASFV genotyping (Giammarioli et al., 2011). The uncharacterized protein B407L shows 93.9% amino acid identity in all isolates except for Malawi Lil-20/1. This protein is identical in E75, L60 and 26544/OG10 isolates and differs from the Benin 97/1 protein by the absence of a duplicated five amino acid "SIRNG" pattern (Fig. 3B). If compared with low virulence isolates OURT 88/3 and NHV, 26544/OG10 presents one "DSICS" repeat at position 102. The tissue culture-adapted isolate BA71V also exhibits these repeats. Comparing all of the genotype I isolates (Benin 97/1, E75, OURT 88/3, 26544/OG10, BA71V, L60 and NHV), Benin 97/1, OURT 88/3 and NHV differ by the presence of one duplicated five amino acid pattern "DSISC". In Benin 97/1, the residue Isoleucine is replaced by Valine. In comparison to the other isolates, only Benin 97/1 displays a "SIRN" repeat located at position 122. Isolate Ken06.Bus lacks 20 residues, from 113 to 132. African isolates Mkuzy 1979, Tengani 62 and Benin 97/1 display "GSIRS" and "GSIRN" repeats that are absent in isolate Ken06.Bus. Ken05/Tk1 shows an entirely different sequence, namely "ESVCSGSMRSG". Transmembrane protein B169L in 26544/OG10 isolate is identical to E75 and L60, whereas it is five amino acids longer in Benin 97/1. The protein from attenuated cell cultured isolate BA71V differs from OURT 88/3 and NHV by the presence of one duplicated pattern "PAGPK". The same protein differs from Georgia 2007/1 isolate by the presence of "AGPKP" and three amino acid substitutions. The pL60L protein from the BA71V and Georgia 2007/1 isolates is smaller than in isolate 26544/OG10 that fully matches the other genotype I isolates: Benin 97/1, E75, L60, NHV and OURT 88/3 with a 100% identity. The pL60L protein of BA71V differ from these isolates by the insertion of the nine residues "SQNTDLQNTE", whereas in Ken06.Bus this protein is 73 amino acids larger than other nine isolates under scrutiny.

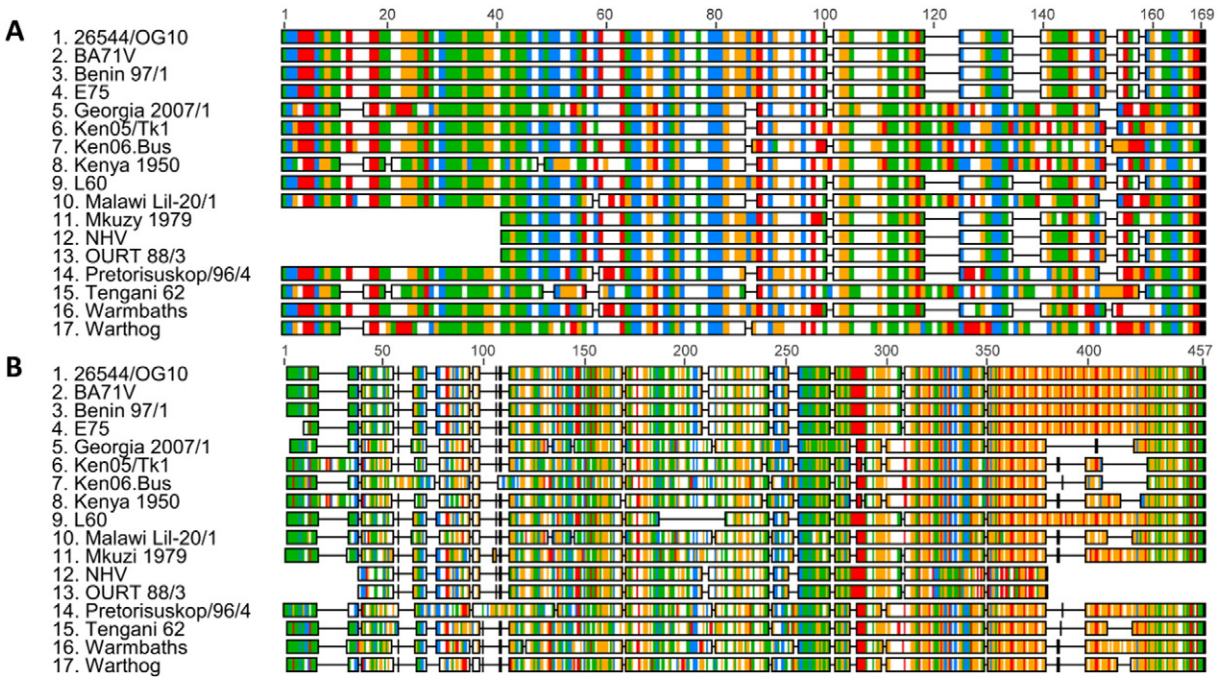

Fig. 2. Protein alignment of EP153R (A) and EP402R (B) in all isolates.

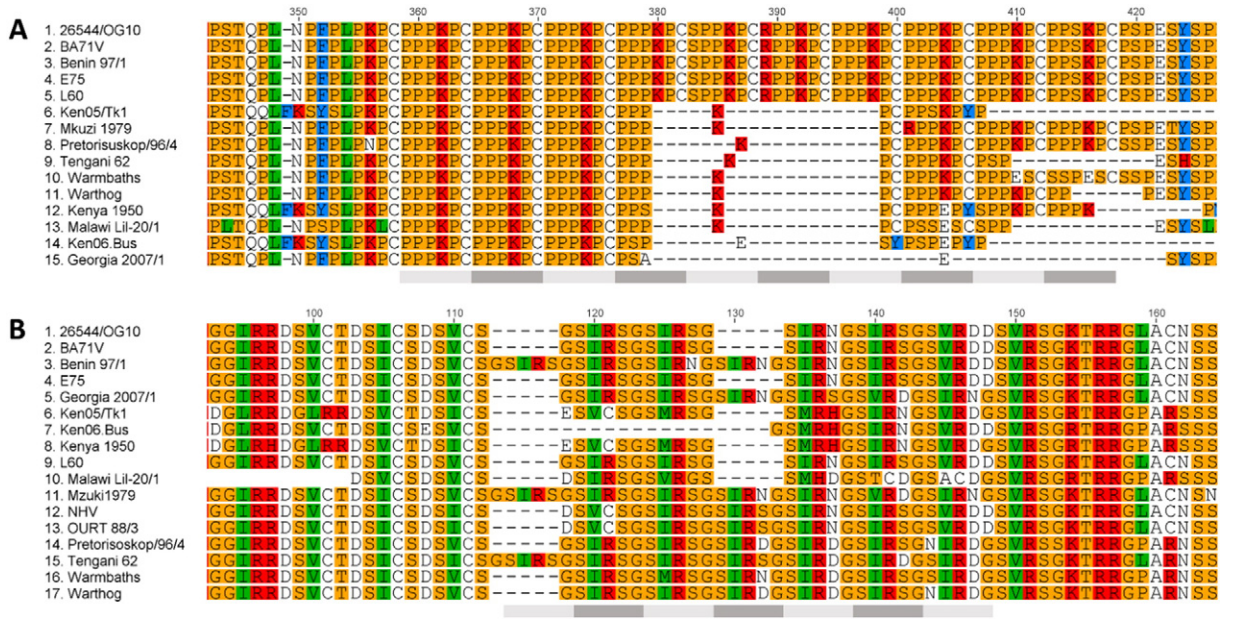

Fig. 3. Detail of the protein alignment of EP402R showing the tandem repeats (grey bars) in the 15 isolates aligned in this region (A). Detail of the protein alignment of B407L showing the tandem repeats (grey bars) (B).

3.4. Diverging intergenic regions

The 26544/OG10 genome sequence displays variable intergenic sequences when compared to other genomes, such as the region between E111R and E66L (both genes coding for an uncharacterized protein). Another hypervariable region extends between two gene sequences codifying for L-transcription factor S-II-related protein and uncharacterized protein I73R. One more divergent intergenic sequence is located between I73R and I329L, coding for transmembrane protein I329L. The intergenic region ranging from MGF100-2L and MGF100-3L features a 17 bp deletion both in 26544/OG10 and in L60; such deletion is not found in the Benin 97/1 isolate. Several hypervariable intergenic regions can be observed, such as the region located between B475L and B354L, as well as the region between CP123L and CP2475L. An extremely hypervariable intergenic region is located between DP96R and MGF 360-19R.

3.5. Multigene family

Complete genome comparison shows that most variation is located at the left end of the genomes and ensues from the presence or absence of different members of the MGFs (Neilan et al., 2002; Zsak et al., 2001). Some authors proved that both BA71V and OURT 88/3 isolates are missing a sequence equaling to 10 kbp, from a region located 20 kbp from the left end of the genome (Chapman et al., 2008). We found that this region is also present in the 26544/OG10 genome and exhibits a high level of similarity to L60 and Benin 97/1. Some ORF deletions are observed close to the right genome end, notably between the tissue culture-adapted BA71V isolate and other isolates, including Georgia 2007/1. The isolates showing the highest sequence divergence to the Georgia 2007/1 are Kenya 1950 and Malawi Lil-20/1. The 26544/OG10 isolate has 27 MGF members; two members of MGF 100 (MGF100-2l and 3L) and only five members of MGF 110 (MGF 110 1L-11L-12L-13L-14L) in comparison with other genomes such as Georgia 2007/1, which has 12 of the 14 known members of MGF 110. The MGF110 13L is partially fused with MGF 110 14L. It can be inferred that MGF360-2L in 26544/OG10 is 153 bp shorter than Benin 97/1, as the insertion of a cytosine at position 1589 entails a stop codon resulting in a truncated protein.

The comparison of avirulent genomes OURT 88/3 and NHV to the 26544/OG10 isolate highlights the absence of members belonging to MGF100 1R, MGF 1102L, 4L, 5L and 9L. The 26544/OG10 genome contains all of the four known members of MGF 300 (MGF1L-2R-3L and 4L). This isolate contains 16 of the 18 known members of the MGF360, in contrast with avirulent isolate genomes, which have only 10 of the MGF360, lacking 6L-10L-11L-12L-13L-14L but containing MGF360-17R (absent in all of the virulent isolates). The 26544/OG10 isolate and seven other isolates - including Benin 97/1, Georgia 2007/1 and five African isolates - contain 10 of the 11 MGF 505 members identified. Tissue culture-adapted isolate BA71V, non-pathogenic isolate NHV and OURT 88/3 lack one (MGF 505/530-1R) or two (MGF 505/530-1R and 2R) of the MGFs (Chapman et al., 2008). Further investigation into the role of individual members of the five MGFs on interferon response is ongoing (Dixon et al., 2013).

3.6. Comparison between 26544/OG10 and avirulent isolates

The global alignment identity between 26544/OG10 and OURT 88/3 is 91.0%, the same between 26544/OG10 and NHV, whereas identity against BA71V equals 99.2%. BA71V shows a 266 bp gap from 182,311 to 184,976 bp compared to 26544/OG10, OURT 88/3 and NHV. BA71V shows a single macrodeletion in the A528R gene, replaced with MGF-505-6R and 7R in the 26544/OG10 genome and with MGF 505-7R and 8R in the OURT 88/3 and NHV. The main difference between 26544/OG10 and BA71V is the absence of MGF 360 10L, MGF 360-11L, MGF 505-1R and MGF 360-12L,13L,14L in the left region of BA71V. Moreover, in the A528R gene the BA71V genome displays a smaller deletion than in 26544/OG10. Compared to 26544/OG10, BA71V complete genome shows a 2667 bp deletion. This deletion starts at position 163,861 bp in the right region on the BA71V genome and results in the loss of five ORFs. Similarly to BA71V, a larger deletion of approximately 4.5 Kb in the variable left region of the 26544/OG10 genome leads to loss or truncation in the MGF 110-13L gene.

3.7. Gene Ontology

The ten clusters identified in 26544/OG10, OURT 88/3, Georgia 2007/1 and NHV genes are transmembrane region, nucleotide-binding, nucleotide phosphate-binding region, host cytoplasm, transcription, metal-binding, signal peptide, apoptosis, host nucleus and DNA repair. These clusters indicate that between the four isolates there are no differences in the terms, just irrelevant value of enrichment score (Supplementary Table S1).

3.8. Phylogenetic analysis

In order to determine the genetic relationship between the ASFV isolates, we performed multiple sequence alignments of the whole genome sequences from the 17 ASFV isolates. The comparison of the 26544/OG10 isolate genome nucleotide sequence to other 16 isolates indicated that this is most closely related to isolates Benin 97/1, E75 and L60 (Fig. 1). The phylogenetic analysis showed that the Sardinian isolate 26544/OG10 clusters with other genotype I isolates such as Benin 97/1, E75 and L60 (Fig. 4). Isolates BA71V, OURT 88/3, NHV, from the same genotype, cluster in divergent subgroups. Other European isolates (Georgia 2007/1), Southern (Mkuzi 1979, Tengani 62, Warmbaths, Warthog and Pretorisuskop/96/4) and Eastern Africa isolates (Malawi Lil-20/1, Kenya 1950, Ken05/Tk1 and Ken06.Bus) form two separate and more distantly related clusters. Phylogenetic trees for six divergent proteins are shown in Fig. 5. The EP402R, X69R, EP153R, DP96R, I10L and L60L protein sequences from the 26544/OG10 isolate cluster more closely with the L60, Benin 97/1 and E75 isolates; minor variations in the phylogenetic analysis of individual proteins that are more divergent in 26544/OG10 does not always match the clustering observed by comparing the whole genome alignment (Fig. 1).

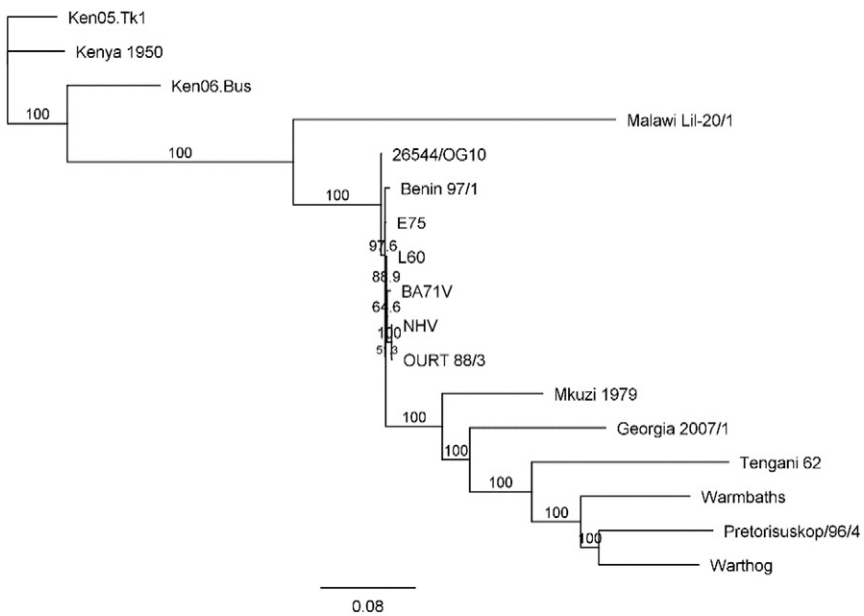

Fig. 4. Neighbor-Joining phylogenetic tree constructed from a multiple nucleotide sequence alignment of 17 isolates.

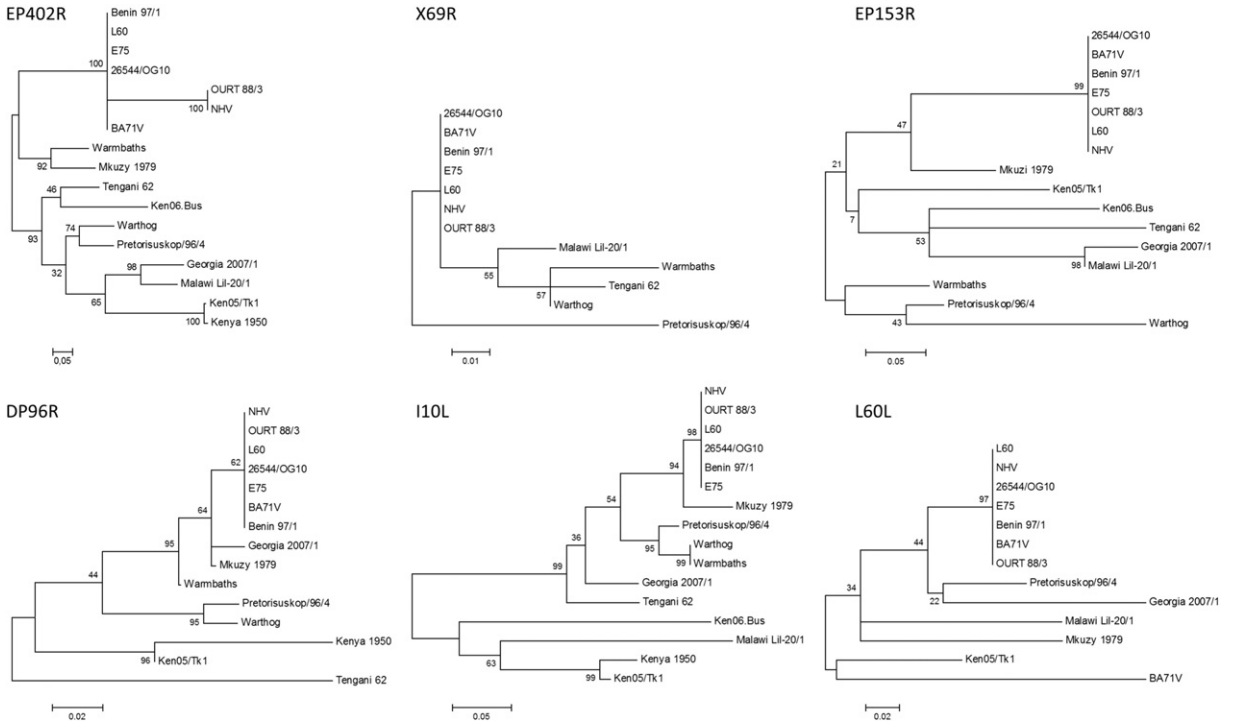

Fig. 5. Phylogenetic comparison of six of the most divergent African swine fever virus proteins. CD2-like protein EP402R, uncharacterized membrane protein X69R, lectin-like EP153R, uncharacterized protein DP96R, I10L P22 and A-F L60L.

4. Discussion

In this work we analyzed the full genome sequence of the strain 26544/OG10, a virulent, hemadsorbing virus obtained from tissue samples from pig with clinical symptoms of ASF, selected among the viruses that have been circulating in Sardinia since 1990. The complete genome was compared to the 16 ASFV genomes available in the database. Comparative genomic analysis aims to underscore genetic assortment diversification in distinct viral isolates, to identify deletions (SNPs) and to carry out evolutionary studies, where results are also integrated by testing alternative targets and gene regions in addition to those used for phylogenetic purposes to date. Alignments performed between the sequence of Sardinian isolate and the other complete genome sequences of ASFV highlights the higher match between the strain 26544/OG10 and L60, Benin 97/1, BA71V (99.2% identity) and a variable identity percentage with the other p72 genotype I strains. The comparative analysis of the Sardinian isolate showed many differences in a great number of genes, 43 of which are still uncharacterized. The most divergence ORFs encoding for unknown function proteins such as DP96R and X69R. Further interesting differences concern hypervariable ORFs (B602L, EP402R, B407L). The CD2v protein EP402R is identical between Sardinian isolate, Benin 97/1 and BA71V but is shorter in other strains belonging to p72 genotype I. Variations were found in the C-terminal region of the CD2v protein containing a variable number of tandem repeats sequences between isolates (Sanna et al., 2016). The B602L protein also presents tandem repetitive amino acids sequences and 88% of identity with other isolates. The biological significance of these variation in tandem repeats is not clear, certainly they maintain the reading frame and the contraction or expansion in protein structures does not interfere with protein functions and virus virulence. As stated before the virus circulating in Sardinia has undergone genetic variation in two genome regions, B602L and EP402R. This variant has rapidly replaced the oldest viruses perhaps due to some selective advantage although clinical data suggest that Sardinian ASFV has never changed its virulence.

Other differences between ASFV genomes were found in the left and right variable regions, mostly in the MGF 110 and 360 families, in relation to number of copies and gene sequence. This ensues from comparing the Sardinian isolate genome to the avirulent isolates NHV, OURT 88/3 and BA71V, where the absence of seven members of MGF 110 and 6 members of MGF 360 (6L-10L-11L-12L-13L-14L) can be observed. Although the presence MGF 110 members seems not to be essential for replication or to have a role in virulence (Aguero et al., 1990), several genes encode proteins with signal peptides and cleavage sites suggesting that they are secreted from infected cell (Dixon et al., 2013). Members of MGF 360 members have been implicated in replication in macrophages, virus replication in ticks and IFN I immune response (Afonso et al., 2004; Burrage et al., 2004; Neilan et al., 2002). MGF 360 – 6L may constitute another virulence factor but its function still lacks characterization.

Considering the emergency still underway in Sardinia and the high risk of spread in whole Europe since the virus introduction in Poland in 2014, the development of a highly effective vaccine within a short term would be a beneficial achievement. Despite intensive research toward the identification of marker vaccines against ASF, these were only found to confer partial protection in

vaccinated animals (Argilaguet et al., 2011; Guinat et al., 2015; O'Donnell et al., 2015). Such failure is mainly due to the inability of the host antibodies to completely neutralize the ASFV infection. Further studies are therefore needed to develop effective next generation immunizing pharmaceuticals.

The new information obtained in this work provides novel research targets for future studies in order to improve molecular features of ASFV circulating in Sardinia and may lead to select some epitopes for use as successful vaccine candidate and to identify possible target of antiviral compounds.

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