

Table S1: List of primers used in this study for deletion detection.

Forward		Reverse		Start	Stop	Estimated Size
ASFV1-DEL_F	CAGACGTTGCCTATTCGG	ASFV1-DEL_R	GCTGAGAGACAATTTGCG	11386	12074	688
ASFV2-DEL_F	CCGCAAATTGTCTCTCAG	ASFV2-DEL_R	GCTCTGACGTTGACAGCT	12056	12994	938
ASFV3-DEL_F	GTCTTTCGTCCTTTCCG	ASFV3-DEL_R	GGACACCAGTGAACCTG	12866	12315	551
ASFV4-DEL_F	GCTGTCAACGTCAGAGCA	ASFV4-DEL_R	CACCAGTGAACCTGTTTC	12964	13294	330
ASFV5-DEL_F	AAACAGGTTCACTGGTGTC	ASFV5-DEL_R	GTCCAGATAAGCCTGACAT	13294	14241	947
ASFV6-DEL_F	GATGTCAGGCTTATCTGGA	ASFV6-DEL_R	ACGGACGTTGTTATCCTGG	14222	15042	820
ASFV7-DEL_F	GCTCCCCAGGATAACAAC	ASFV7-DEL_R	GCCATTGCTTTAGCATCT	15019	15569	550
ASFV8-DEL_F	CTGTACAGATGCTAAAGCAA	ASFV8-DEL_R	ACATATTTACATCCGTGGC	15546	16400	854
ASFVfullDEL_F	CAGACGTTGCCTATTCGG	ASFVfullDEL_R	ACATATTTACATCCGTGGC	11386	16400	5014

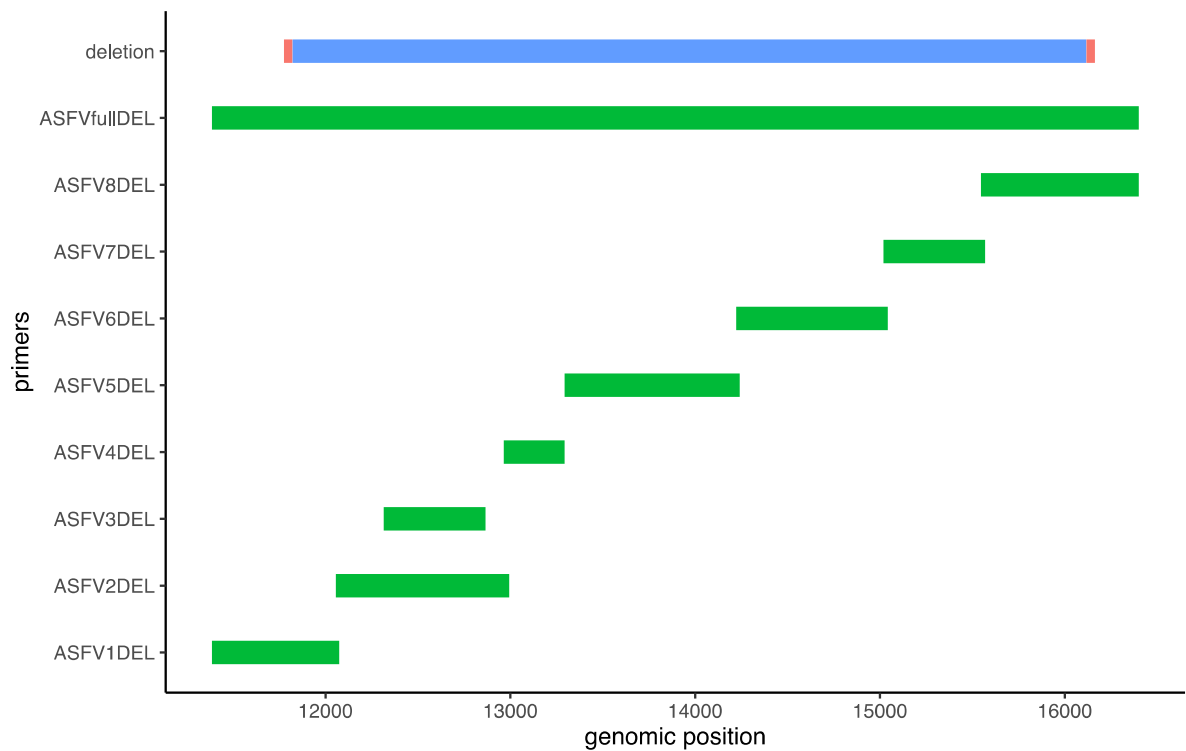


Figure S1. Primer locations along the ASFV genome with respect to the reference sequence KX354450. The choice of primers (green bars) was driven by the putative location of the deletion. The location of the deletion (blue bar) and of the regions of perfect microhomology (in red) is shown at the top.

Table S2: Metadata associated with ASFV whole genomes analyzed in this study. GenBank accession number and tip labels used in the phylogenetic trees are also reported.

SeqID	SeqName	SampleID	GenBank	Host	Sampling	Country	City
PRT LISB 1960	PRT LISB 1960	L60	NC044941	Domestic Pig	1960	Portugal	Lisbon
ESP 1975	ESP 1975	E75	NC044958	Domestic Pig	1975	Spain	
ITA CA 1 1978	ITA CA 1 1978	56/Ca/1978	MN270969	Domestic Pig	1978	Italy	Cagliari
ITA CA 2 1978	ITA CA 2 1978	CA1978_2	MW723480	Domestic Pig	1978	Italy	Cagliari
ITA NU 1979	ITA NU 1979	NU1979	MW723481	Domestic Pig	1979	Italy	Nuoro
ITA CA 1979	ITA CA 1979	57/Ca/1979	MN270970	Domestic Pig	1979	Italy	Cagliari
ITA SS 1981	ITA SS 1981	SS1981	MW788409	Domestic Pig	1981	Italy	Sassari
ITA NU 1 1981	ITA NU 1 1981	139/Nu/1981	MN270971	Domestic Pig	1981	Italy	Sassari
ITA NU 2 1981	ITA NU 2 1981	NU1981_2	SRR13976567	Domestic Pig	1981	Italy	Nuoro
ITA TO 1983	ITA TO 1983	ITALY1983	SRR13976568	Domestic Pig	1983	Italy	Torino
ITA OR 1984	ITA OR 1984	OR1984	MW800838	Domestic Pig	1984	Italy	Oristano
ITA OR 1985	ITA OR 1985	140/Or/1985	MN270972	Domestic Pig	1985	Italy	Oristano
ITA CA 1985	ITA CA 1985	85/Ca/1985	MN270973	Domestic Pig	1985	Italy	Cagliari
ITA NU 1986	ITA NU 1986	NU1986	MW723482	Domestic Pig	1986	Italy	Nuoro
ITA NU 1 1990	ITA NU 1 1990	NU1990_1	MW723483	Domestic Pig	1990	Italy	Nuoro
ITA NU 2 1990	ITA NU 2 1990	NU1990_2	SRR13976561	Domestic Pig	1990	Italy	Nuoro
ITA NU 3 1990	ITA NU 3 1990	141/Nu/1990	MN270974	Domestic Pig	1990	Italy	Nuoro
ITA NU 2 1991	ITA NU 2 1991	NU1991_2	MW723484	Domestic Pig	1991	Italy	Nuoro
ITA NU 3 1991	ITA NU 3 1991	NU1991_3	MW723485	Domestic Pig	1991	Italy	Nuoro
ITA NU 7 1991	ITA NU 7 1991	NU1991_7	MW723486	Domestic Pig	1991	Italy	Nuoro
ITA NU 9 1991	ITA NU 9 1991	NU1991_9	SRR13976566	Domestic Pig	1991	Italy	Nuoro
ITA NU 1993-07-1	ITA NU Jul-1993	NU1993_2	MW723488	Domestic Pig	01/07/93	Italy	Nuoro
ITA OR 1993	ITA OR 1993	OR1993_1	MW723487	Domestic Pig	1993	Italy	Oristano
ITA NU 1 1995	ITA NU 1 1995	142/Nu/1995	MN270975	Domestic Pig	1995	Italy	Nuoro
ITA NU 2 1995	ITA NU 2 1995	NU1995_2	MW723489	Domestic Pig	1995	Italy	Nuoro
ITA NU 3 1995	ITA NU 3 1995	NU1995_3	MW723490	Domestic Pig	1995	Italy	Nuoro
ITA NU 4 1995	ITA NU 4 1995	NU1995_4	MW723491	Domestic Pig	1995	Italy	Nuoro
BEN 1997	BEN 1997	BENIN_1997	NC044956	Domestic Pig	1997	Benin	
ITA TEM 2002-04-19	ITA TEM Apr-2002	24225_2002	MW788411	Domestic Pig	19/04/02	Italy	Tempio
ITA ORU 2004-07-7	ITA ORU Jul-2004	44076_2004	MW723500	Domestic Pig	07/07/04	Italy	Orune
ITA MAC 2004-07-12	ITA MAC Jul-2004	45539_2004	SRR13976563	Domestic Pig	12/07/04	Italy	Macomer
ITA BUL 2004-10-5	ITA BUL Oct-2004	26/Ss/2004	MN270977	Domestic Pig	05/10/04	Italy	Bultei
ITA TEL 2004-11-26	ITA TEL Nov-2004	74377_2004	MW723496	Domestic Pig	26/11/04	Italy	Telti

ITA SAD 2005-05-5	ITA SAD May-2005	22649_2005	MW723497	Domestic Pig	05/05/05	Italy	Sadali
ITA PAL 2005-12-1	ITA PAL Dec-2005	72398WB_2005	MW723495	Wild boar	01/12/05	Italy	Palau
ITA TER 2007-11-23	ITA TER Nov-2007	72407_2007	MN270978	Domestic Pig	23/11/07	Italy	Tergu
ITA URZ 2007-03-11	ITA URZ Mar-2007	72912WB_2007	MW723498	Wild boar	11/03/07	Italy	Urzulei
ITA URZ 2008-01-6	ITA URZ Jan-2008	1537WB_2008	MW788405	Wild boar	06/01/08	Italy	Urzulei
ITA TAL 2008-01-20	ITA TAL Jan-2008	4996WB_2008	MW723492	Wild boar	20/01/08	Italy	Talana
ITA BEN 2008-04-10	ITA BEN 1 Apr-2008	22137_2008	MW723499	Domestic Pig	10/04/08	Italy	Benetutti
ITA VIL 2008-04-16	ITA VIL 1 Apr-2008	22943_2008	MW788406	Domestic Pig	16/04/08	Italy	Villasor
ITA VIL 2008-04-17	ITA VIL 2 Apr-2008	23221_2008	MW723494	Domestic Pig	17/04/08	Italy	Villasor
ITA BEN 2008-04-28	ITA BEN 2 Apr-2008	25185_2008	MW788410	Domestic Pig	28/04/08	Italy	Benetutti
ITA STI 2008-06-1	ITA STI Jun-2008	46830_2008	MW723493	Domestic Pig	01/06/08	Italy	Stintino
ITA STI 2008-09-1	ITA STI Sep-2008	47/Ss/2008	KX354450	Domestic Pig	01/09/08	Italy	Stintino
ITA ORU 2009-01-15	ITA ORU Jan-2009	1628_2009	SRR14601691	Wild boar	15/01/19	Italy	Orune
ITA ONI 2009-05-22	ITA ONI May-2009	28170_2009	SRR13976565	Domestic Pig	22/05/09	Italy	Oniferi
ITA BAU 2010-05-20	ITA BAU May-2010	26544/OG10	KM102979	Domestic Pig	20/05/10	Italy	Baunei
ITA BONO 2011-06-14	ITA BONO Jun-2011	31208_2011	MW736612	Domestic Pig	14/06/11	Italy	Bono
ITA ALA 2012-02-12	ITA ALA Feb-2012	2019WB_2012	MW736598	Wild boar	12/02/12	Italy	Ala dei Sardi
ITA OSC 2012-04-18	ITA OSC Apr-2012	97/Ot/2012	MN270979	Domestic Pig	18/04/12	Italy	Oschiri
ITA BIT 2011-12-30	ITA BIT Dec-2011	63525WB_2011	MW736603	Wild boar	30/12/11	Italy	Bitti
ITA BUD 2013-03-25	ITA BUD Mar-2013	30322_2013	MW736600	Domestic Pig	25/03/13	Italy	Budduso
ITA BON 2013-04-3	ITA BON Apr-2013	32516_2013	MW736607	Domestic Pig	03/04/13	Italy	Bonorva
ITA PAT 2013-05-20	ITA PAT May-2013	47039_2013	MW736597	Domestic Pig	20/05/13	Italy	Pattada
ITA BOL 2013-05-27	ITA BOL May-2013	49179WB_2013	MW736601	Wild boar	27/05/13	Italy	Bolotana
ITA PAT 2013-11-11	ITA PAT Nov-2013	98039_2013	MW736599	Domestic Pig	11/11/13	Italy	Pattada
ITA NUL 2013-12-16	ITA NUL Dec-2013	113049WB_2013	MW736608	Wild boar	16/12/13	Italy	Nulvi
ITA CAS 2014-01-27	ITA CAS Jan-2014	11484WB_2014	SRR13975654	Wild boar	27/01/14	Italy	Castelsardo
ITA VILLA 2014-02-21	ITA VILLA Feb-2014	22653/Ca/2014	MN270980	Domestic Pig	21/02/14	Italy	Villanovatulo
ITA BON 2014-03-31	ITA BON Mar-2014	35479_2014	MW788408	Domestic Pig	31/03/14	Italy	Bonorva
ITA TER 2014-06-3	ITA TER Jun-2014	51268_2014	MW736605	Domestic Pig	03/06/14	Italy	Tergu
ITA BEN 2015-01-14	ITA BEN Jan-2015	6396WB_2015	MW736609	Wild boar	14/01/15	Italy	Benetutti
ITA ORG 2015-02-10	ITA ORG Feb-2015	15998_2015	MW736604	Domestic Pig	10/02/15	Italy	Orgosolo
ITA BON 2015-03-25	ITA BON Mar-2015	28928_2015	MW736610	Domestic Pig	25/03/15	Italy	Bonorva
ITA SEN 2015-04-10	ITA SEN Apr-2015	31479_2015	MW788407	Domestic Pig	10/04/15	Italy	Sennori
ITA ANE 2015-04-21	ITA ANE Apr-2015	33747WB_2015	MW736613	Wild boar	21/04/15	Italy	Anela
ITA SAR 2016-06-16	ITA SAR Jun-2016	53706_2016	MW736602	Domestic Pig	16/06/16	Italy	Sarule

ITA DOL 2017-01-10	ITA DOL Jan-2017	3312_2017	SRR13976564	Domestic Pig	10/01/17	Italy	Dolianova
ITA DES 2017-03-14	ITA DES Mar-2017	34403WB_2017	MW736606	Wild boar	14/03/17	Italy	Desulo
ITA SEUI 2017-05-23	ITA SEUI May-2017	52060_2018	SRR13976569	Free Ranging Pig	23/05/17	Italy	Seui
ITA ARI 2018-01-15	ITA ARI Jan-2018	8343_2018	SRR13976571	Free Ranging Pig	15/01/18	Italy	Aritzo
ITA LOT 2018-06-8	ITA LOT 1 Jun-2018	54684_2018	MW647171	Free Ranging Pig	08/06/18	Italy	Lotzorai
ITA LOT 2018-06-14	ITA LOT 2 Jun-2018	56140_2018	MW736611	Free Ranging Pig	14/06/18	Italy	Lotzorai
ITA DES 2018-06-15	ITA DES Jun-2018	55234_2018	MT932579	Free Ranging Pig	11/06/18	Italy	Desulo
ITA TAL 2018-12-15	ITA TAL Dec-2018	103917_2018	MT932578	Free Ranging Pig	17/12/18	Italy	Talana
ITA LAN 2019-01-14	ITA LAN Jan-2019	7212_2019	ON260838	Wild boar	14/01/19	Italy	Lanusei
ITA PAT 2019-01-14	ITA PAT Jan-2019	7303_2019	ON260839	Wild boar	13/01/19	Italy	Pattada

Table S3: Metadata associated with ASFV microhomology analysis.

Sequence name	Country	Genotype
KX354450.1 African swine fever virus isolate 47/Ss/2008, complete genome	Italy	I
MN270969.1 African swine fever virus isolate 56/Ca/1978, complete genome	Italy	I
NC_044958.1 African swine fever virus E75 complete genome, strain E75	Spain	I
NC_044941.1 African swine fever virus strain L60, complete genome	Portugal	I
MN913970.1 African swine fever virus strain Liv13/33 (OmLF2) isolate OmLF2, complete genome	France	I
NC_044957.1 African swine fever virus OURT 88/3 (avirulent field isolate), complete genome	Portugal	I
U18466.2 African swine fever virus strain BA71V, complete genome	Spain	I
NC_044943.1 African swine fever virus strain NHV, complete genome	Portugal	I
NC_044959.2 African swine fever virus isolate ASFV Georgia 2007/1 genome assembly, complete genome: monopartite	Georgia	II
AY261365.1 African swine fever virus isolate Warmbaths, complete genome	South Africa	III
MN641876.2 African swine fever virus isolate RSA_W1_1999, complete genome	South Africa	IV
AY261361.1 African swine fever virus isolate Malawi Lil-20/1 (1983), complete genome	Malawi	VIII
MH025916.1 African swine fever virus strain R8, complete genome	Uganda	IX
MW856067.1 African swine fever virus strain BUR/18/Rutana, complete genome	Burundi	X
MN641877.2 African swine fever virus isolate RSA_2_2004, complete genome	South Africa	XX
MN336500.3 African swine fever virus isolate RSA_2_2008, complete genome	South Africa	XXII

Table S4: Deletions and changes between the two isolates under study with the reference strain KX354450. Genome position, nucleotide, type of mutation, and predicted features are reported.

Gene	Genome Position	Ref	Alt_7212WB/19	Alt_7303WB/19	Aa_chg	Mutation	Predicted features
IG	11820..12052	no deletion	deletion	deletion			Intergenic region
MGF360-6L	12053..13180	no deletion	deletion	deletion			MGF360 family
IG	13181..14063	no deletion	deletion	deletion			Intergenic region
X69R	14064..14273	no deletion	deletion	deletion			Uncharacterized protein
IG	14274..14459	no deletion	deletion	deletion			Intergenic region

MGF300-1L	14460..15266	no deletion	deletion	deletion			MGF300 family
IG	15267..16162	no deletion	deletion	deletion			Intergenic region
MGF 505-3R (*)	30649	A	G	G	Silent	Point mutation	MGF505 family
IG (*)	34552	T	C	C	NA	Point mutation	Intergenic region
MGF360-15R (*)	45961	T(7)	T(8)	T(8)	Frameshift	INDEL	MGF360 family
URF19 (*)	54920	C	T	T	Y21C	Point mutation	Undefined reading frame (Hypothetical protein)
EP364R (*)	71175	A	G	G	A319T	Point mutation	XPF_NUCLEASE-LIKE
EP364R (*)	71216	A	G	G	Silent	Point mutation	XPF_NUCLEASE-LIKE
URF22 (*)	77296	A(9)	A(10)	A(10)	Frameshift	INDEL	Undefined reading frame (Hypothetical protein)
C315R (*)	83384	T	C	C	Silent	Point mutation	TFIIB-like factor
IG (*)	95173	G	A	A	NA	NA	Intergenic region
IG (*)	110196	G	A	A	NA	NA	Intergenic region
D205R (*)	140311	G	A	A	P131S	Point mutation	RNA polymerase subunit 5
S273R	142703	G	A	A	Silent		Cysteine protease S273R
URF56 (*)	152555	G(5)	G(6)	G(6)	Frameshift		Undefined reading frame (Hypothetical protein)
IG (*)	176705	A(7)	A(8)	A(8)	Na		Intergenic region
IG (*)	179838	C(9)	C(10)	C(10)	Na		Intergenic region

(*): changes in Sardinian ASFV isolates already described [16,50]

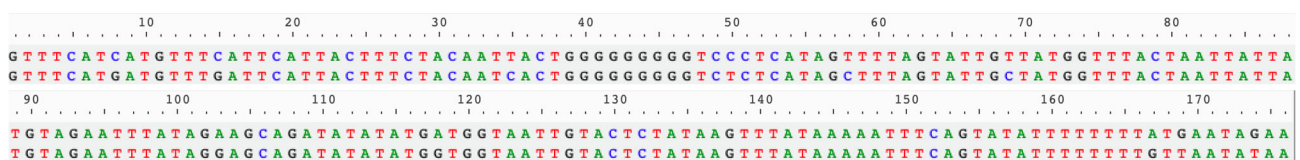


Figure S2. Alignment of the two regions of imperfect microhomology starting at positions 11656 and 15998 of the Sardinian ASFV reference sequence (KX354450).

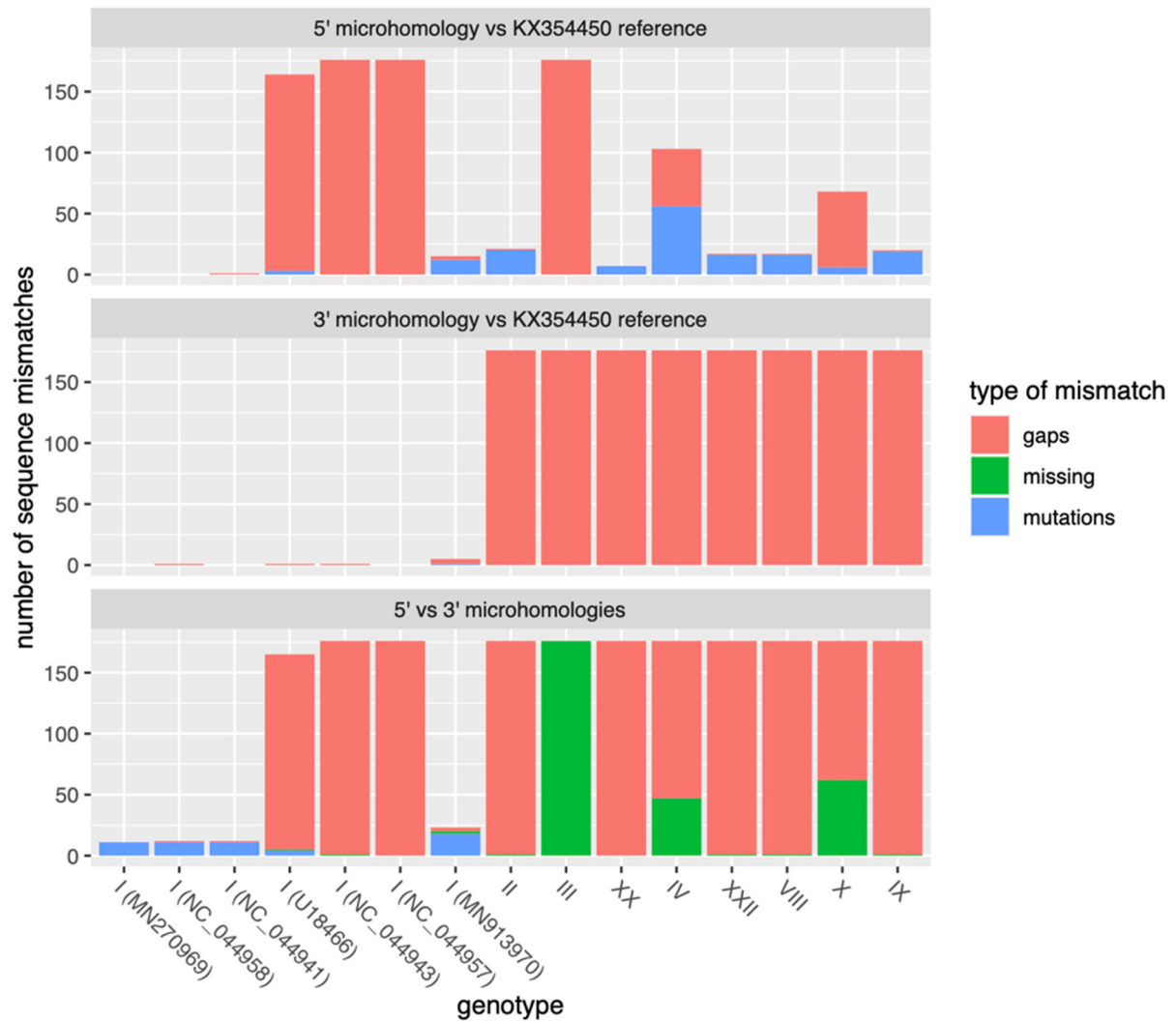


Figure S3. Conservation of the imperfect microhomology and the two 176 bp sequences involved (one at the 5' end of the deletion, one at the 3') among ASFV genotypes. Top: number of mismatches between the sequence closer to the 5' end of the genome in KX354450 and the corresponding sequences in other samples from different genotypes, ordered by genomic divergence from KX354450. Middle: mismatches between the sequence closer to the 3' end of the genome in KX354450 and the corresponding sequences in other samples from different genotypes. Bottom: mismatches between the two sequences from the same sample. "Missing" denotes bases that are missing (i.e. contain gaps in the alignment) for both sequences.