M.V. Nefedeva, I.A. Titov, K.A. Mima, A.S. Malogolovkin

Analysis of the African Swine Fever Virus Immunomodulatory Proteins

Molecular Virology Laboratory Federal Research Center for Virology and Microbiology, 601125, Volginsky, Vladimir region, Russia

For correspondence: Alexander S. Malogolovkin, PhD of Biological Sciences, Head of the Molecular Virology Laboratory Federal Research Center for Virology and Microbiology, Associate Director, E-mail: Malogolovkin@inbox.ru

Information about authors:

Nefedeva Mariia Vladimirovna e-mail: masha67111@mail.ru
https://orcid.org/0000-0002-6143-7199

Titov Ilya Andreevich e-mail: titoffia@yandex.ru
https://orcid.org/0000-0002-5821-8980

Mima Ksenia Aleksandrovna e-mail: mima89@ya.ru
https://orcid.org/0000-0001-7184-6968

Malogolovkin Alexander Sergeevich e-mail: Malogolovkin@inbox.ru
https://orcid.org/0000-0003-1352-1780

Summary

Molecular epidemiology of viral infections traditionally based on the analysis of changes in individual genes or genetic markers. The analysis of the African swine fever virus (ASFV) genes encoding immunomodulatory proteins is an important tool for studying the diversity and evolution of the virus. In this work, we carried out a structural and phylogenetic analysis of the ASF virus immunomodulatory proteins 5EL (*A238l* gene), I14L (*Dp71l* gene), K11L (*I329l* gene). The degree of nucleotide substitutions of the ASFV concatenated genes *A238l*, *I329l* and *Dp71l* revealed purifying (stabilizing) selection at the nucleotide sequences level. The variability characteristic of the selected group of ASFV genes is of great interest for the genetic differences search in immunomodulatory proteins. The sequencing results of the *A238l*, *I329l* and *Dp71l* genes and their phylogenetic analysis showed that these genes are

conservative among a large group of ASFV genes. The *I329l* gene is a genetic marker of common origin. The East African strains (Genotype X) of *Dp71l* gene have two forms: a long (184 amino acids) and a short (from 70 to 72 amino acids) and is formed by fusion of the 13L and 14L. All ASF virus Russian isolates isolated in 2016-2017 were identical to the reference strain ASFV/Georgia/wb/2007. Characterization of variability 5EL protein, I14L, K11L may be serve to identify target sites in the ASFV genome and to develop vaccines. The obtained data allow to evaluate the genetic diversity of the ASFV immunomodulatory proteins and the dynamics of their evolution, to predict the possible participation of the *A238l*, *I329l* and *Dp71l* genes in the virulence of various ASFV strains.

Keywords: African swine fever virus, sequencing, phylogenetic analysis, immunomodulating proteins, analysis of synonymous and nonsynonymous substitutions.

Introduction

African swine fever (ASF) is a hemorrhagic deadly disease of domestic pigs and boars caused by the complex shell deoxivirus of the *Asfarviridae* family. Mortality in infected herds reaches 100%. The disease is transmitted from sick animals and virus carriers by alimentary, contact and transplacental [1]. The ASFV genome is a linear double-stranded DNA molecule with covalently closed ends and terminal inverted repeats (TIR) [2].

Domestic pigs of all age groups and breeds and wild boars are susceptible to the ASFV. Among the African members of the *Suidae* family, wild African and bush pigs, warthogs, may be infected with ASFV but without showing clinical signs. The main virus reservoir in the wild in East African countries are soft mites of the genus *Ornithodoros* [3].

56 Depending on the virus strain, virulence and mode of infection, the observed
57 clinical signs are diverse in a peracute, acute, subacute and chronic forms of infection
58 [4]. The ASF epidemiology is very complex and varies depending on the geographical
59 features of the area and susceptible species of animals (wild boar, domestic pigs, ticks)
60 [3].

61 Effective means of protection against the ASFV has not yet been developed.
62 Historical attempts to protect animals with inactivated vaccines have either failed or
63 yielded conflicting results. Studies using attenuated vaccines have demonstrated their
64 potential to protect pigs from experimental infection with a homologous virulent virus,
65 but rarely against heterologous viruses [5–7]. The study of antigenic diversity among
66 naturally occurring of ASFV isolates is of great interest for vaccine development [8].

67 Recent studies indicate that the virus genetic diversity determined by sequencing
68 the major capsid protein P72 (*B646l* gene) is highest in Central and East Africa [3]. The
69 high level of the ASFV genetic variability between different isolates is explained by the
70 difference in the genome size, but most genetic variation is due to changes in the
71 number and sequence of multigenic families members (MGF) located at both ends of
72 the genome, where they limit the left and right variable regions (LVR and RVR,
73 respectively) [9].

74 The ASFV genome contains a set of genes encoding the proteins responsible for
75 the immune evasion mechanisms. The modulation activity range of viral proteins is very
76 diverse and includes: inhibition of humoral response, interference with interferons,
77 inhibition of cytokines and chemokines, evasion of cytotoxic T lymphocytes (CTLs)
78 and natural killer cells (NKs), and control of the major histocompatibility complex
79 (MHC I and II) function, changes in the effector function of dendritic cells and
80 inhibition of apoptosis [10].

81 The ASFV is a macrophage-tropic virus and can manipulate both innate and
82 adaptive immune response by modulating macrophages functions. According to L.
83 Dixon et al., several ASFV proteins have been identified which prevent the
84 development of the host's immune defense [11]. These include the *A238l* gene that
85 encodes a 5EL protein which acts to inhibit transcriptional activation of host
86 immunomodulatory genes, K11L protein (*I329l* gene) acting as inhibitor of Toll-like
87 receptors signaling pathways and I14L protein (*Dp71l* gene) similar to the herpes
88 simplex virus-encoded virulence factor ICP34.5 which regulates the phosphatase
89 activity of the host cell during infection displacing inhibitory substrates from PP1 host
90 phosphatase and determining the range of sensitive hosts [12—14]. In addition, *A238l*
91 inhibits the host calceneurin dependent pathways by directly binding calceneurin
92 phosphatase [14].

93 However, not all known ASFV isolates have the same immunomodulatory
94 activity [15]. The genetic background of these differences remains unclear. Thus, the
95 detection of genetic markers of evolutionary variability in ASFV immunomodulating
96 proteins will allow to study the ASFV immune evasion mechanisms.

97 The aim of the study is to conduct a comparative analysis of nucleotide
98 sequences of ASFV *A238l, I329l* and *Dp71l* genes involved in immune evasion.

99

100 ## Material and methods

101 At the first stage, the 5EL, K11L and I14L proteins variability was analyzed
102 using the web service Protein Variability Server by Simpson method
103 (http://www.expasy.org/proteomics) [16]. Region for which the values obtained
104 exceeded the threshold value (0.46) were considered variable. SignalP 4.1 web server
105 was used to predict the presence and location of signal peptide cleavage sites in amino
106 acid sequences. (http://www.cbs.dtu.dk/services/SignalP-4.1) [17]. The CCTOP

107 (Constrained Consensus TOPology prediction server) web application provided
108 prediction of the transmembrane protein topology (http://cctop.enzim.ttk.mta.hu) [18].
109 At the next stage of work, 12 isolates isolated from different regions of the
110 Russian Federation in 2016 and 15 strains belonging to different genotypes [19, 20] and
111 serotypes of the ASFV [21] were selected from the State Collection of Microorganisms
112 of the FRCVM. For each seroimmunotype, attenuated and virulent ASFV strains are
113 used as indicated in the **table**.
114 Selection of primers for the copies amplification of the *A238l, I329l* and *Dp71l*
115 genes was carried out by comparing and analyzing the nucleotide sequences of these
116 genes from strains and various ASFV isolates published in the international GenBank
117 database (http://www.ncbi.nlm.nih.gov/) using BioEdit 7.0.1, Oligo 6.0. and the IDT
118 DNA web server (https://eu.idtdna.com/site).
119 DNA extraction from blood and organ suspension was performed using DNA-
120 sorb-B kit («Interlabservice», Russia) in accordance with the manufacturer's
121 instructions. Thermal cycler T100 («Bio-Rad Laboratories», USA) was used to optimize
122 the temperature regimes of PCR and gene sequences amplification.
123 Analysis of the reaction products was carried out by electrophoresis in 1.5%
124 agarose gel and taken into account on ChemiDoc MP («Bio-Rad Laboratories», USA)
125 by detection specific bands in the tracks with test samples relative to the molecular
126 weight marker fragment and the calculated length of the PCR product. PCR products
127 purification from agarose gel was performed by QIAquick Gel Extraction (QIAGEN)
128 and Cleanup - standard («Evrogen», Russia) commercial kit. The sequencing reaction
129 was performed using the Bigdye terminator v3.1 cycle sequencing kit («Applied
130 Biosystems», USA). Genetic Analyzer 3130 sequencer ("Applied Biosystems", USA)
131 was used for sequencing.

132 Multiple alignment of ASFV gene sequences was performed using variants of
133 the ClustalW method and MUSCLE [22]. To construct phylogenetic trees, the
134 maximum likelihood method was used with an additional bootstrap - analysis of 1000
135 random samples using the Mega 6.0 program [23].

136 For the analysis of synonymous (synonymous distance, dS) and non-
137 synonymous substitutions (non-synonymous distance, dN) per site in the *A238l, I329l*
138 and *Dp71l* genes, as well as the number of potentially synonymous and non-
139 synonymous sites for each codon based on the hypothesis of equal frequencies of all
140 nucleotide substitutions, the SNAP (Synonymous Non-synonymous Analysis Program)
141 software package was used (www.hiv.lanl.gov).

142

143 Results and discussion

144 The results of the proteins 5EL, I14L and K11L variability analysis of different
145 ASFV strains showed that the 5EL protein has 13 variable sites, some of which are
146 located in the region of IκB - a similar domain consisting of 3 ankyrin repeats (from 87
147 to 178 amino acids) and C - terminal end of the PxIxITxC/S motif, which binds the
148 catalytic subunit of calcineurin - serintreonin phosphatase (from 200 to 213 amino
149 acids) **(fig. 1).** The ankyrin repetitive proteins although absent in most viruses, are also
150 common among poxviruses. Conservative amino acid residues of ankyrins are crucial
151 for folding and protein stability, and are also involved in the attachment of other
152 proteins to different parts of the cell membrane [24].

153 Analysis of the I14L protein shows that this protein is conservative among the
154 different ASFV strains, but has 7 variable sites. All proteins encoded by this gene
155 contain a central region with a highly conserved 56-amino acid domain and a PP1c_bdg
156 motif (from 7 to 57 amino acids in short form and from 123 to 170 amino acids in long
157 form) of the regulatory subunits (15A and 15B) of protein phosphatase 1, located at the

158 carboxyl end, common to MyD116 and ICP34.5. This conserved C-terminus appears to
159 be the binding region of the catalytic subunit of protein phosphatase-1 (PP1C), which is
160 found in both the herpes simplex virus and in mice and humans [25].

161 The highly glycosylated K11L protein expressed in the cell membrane and on its
162 surface, containing leucine-rich repeats similar to TLR3, is conservative and has no
163 pronounced variability. For him, there are only 8 variable sites, some of which fall into
164 the extracellular topological domain (from 17 to 239 amino acid), as well as the
165 intracellular topological domain (from 261 to 329 amino acid). The transmembrane
166 region of the protein is located at the C-terminus (from 240 to 260 amino acids) **(see fig.**
167 **1).**

168 As a result of observations D. Chapman et al. the 5EL and I14L proteins are
169 attributed to variable, in the work of V. de Oliveira et al. the K11L protein is
170 conservative, and in the works of J. Neilan et al. and C. Abrams et al., the 5EL protein,
171 on the contrary, was recognized as conservative [12, 26—28]. According to the results
172 of our studies **(see fig. 1),** the studied proteins of the ASFV are conservative, especially
173 in comparison with other ASFV variable proteins *(*CD2V, P54*)* [29].

174 In order to determine the genetic relationship at the nucleotide sequences level
175 between ASFV strains and isolates, we performed sequencing and multiple alignment of
176 the concatenated set of *A238l, I329l* and *Dp71l* genes.

177 According to the sequencing results of the *A238l* and *I329l* genes of ASFV
178 isolates, which were isolated on the Russian Federation territory in 2016, are identical to
179 the Georgia_2007/1 parent strain, which is representative of the genotype II.

180 According to the results obtained for the *I329l* gene, we can divide the existing
181 isolates into 5 main clusters, which correlate with their genotypes **(fig. 2).** The first
182 cluster includes strains from South-East Africa of genotype V, seroimmunotypes 3 and
183 7, and full-genome isolates Warthog and Pretorisuskop/96/4 genotypes XIX and XX,

184 seroimmunotype 2. The second cluster consists of the representatives genotype II
185 seroimmunotype 8 from the Russian Federation. A separate cluster contains strains of
186 genotype I seroimmunotype 2. The fourth cluster consists of strains and isolates of
187 European origin seroimmunotypes 1 and 4, with the exception of isolate Benin 97/1 of
188 African origin belonging to genotype I. The fifth cluster included strains and isolates of
189 genotype X isolated on the territory of Tanzania and Kenya, as well as some
190 representatives of genotype VIII and IX. Isolates from South Africa and Portugal with
191 indeterminate and seroimmunotype 2 were not included in any of the groups of listed
192 clusters.

193 An interesting fact is that in 6 East African strains of the genotype X (TKF,
194 Nanyuki, TSP-80, TSP-80/300, TS-7, TS-7/27-230), as in the full-genome isolates
195 Ken05/Tk1, Kenya 1950, Malawi Lil-20/1 and Ken06.Bus strain, the *Dp71l* gene is
196 formed by fusing 13L and 14L. For example, the results of the detection of 2 forms
197 amplification products of the *Dp71l* gene are shown in **fig.3.** The results of our studies
198 confirm the previously obtained data R. Bishop et al. [30].

199 Earlier studies have shown that the method of concatenated genes can resolve
200 ambiguities in phylogenetic constructions based on individual genes. The concatenation
201 of large multigene data sets to improve the accuracy of phylogenetic inference is an
202 accepted technique for clusters consisting of orthologous genes [3].

203 Concatenation is widely used in taxa in which there are complete genomes, for
204 example, in prokaryotes [31] and taxa with small organelle genomes, such as animal
205 mitochondria [32]. The sequence concatenation can hide the main tree of species with
206 different phylogenetic signals in the evolution of individual genes [3].

207 In our work, the amino acid sequences of three proteins 5EL, K11L, and I14L
208 from 32 ASFV strains were concatenated into one pseudosequence and were used to

calculate the ratio of non-synonymous (dN) - synonymous substitution (dS) per site (dN/dS ratio).

The number of synonymous substitutions on the synonymous site prevailed over the number of non-synonymous to a non-synonymous site 0.2788>0.0678 (dS> dN, pS> pN). When calculating the ratio dN/dS, the value is 0.2432<1. This indicates purifying (stabilizing) selection at the level of nucleotide sequences.

Purifying selection occurs most frequently and is characteristic of nucleotide sequences encoding structurally functionally formed proteins. A comparison of the rates of synonymous and nonsynonymous substitutions for ASFV genes revealed from 14 to 18 genes that are subject to positive selection [26]. The role of the immune system in relation to sequence variability can influence the sequences evolution of the ASFV some genes, which therefore can lead to a shift in the time to most recent common ancestor (TMRCA). Lower rates of non-synonymous changes are found in viruses transmitted by arthropod vectors, which reflects the increased cleansing selection pressure associated with replication in various host types. It is possible that many mutations that occur inside the host are removed during transmission between them due to the strong purifying selection, primarily due to the mismatch of the cytotoxic host immune response carried out by T-lymphocytes.

In the work of E. de Villiers et al. 4 models of codon replacement (M1, M2, M7 and M8) were investigated. The M2 model identified 14 ASFV genes under positive selection. These include proteins of the 360 and 505 multigenic families, several hypothetical proteins, a CD2v homologue, several enzymes and viral chaperone *B602l* which ensures the correct folding of the basic protein capsid P72. The strictest model for positive selection M8 identified eighteen genes, eight of which are under positive selection and are genes that can participate in modulating the host cell functions [3].

234 Data analysis of the ratio of non-synonymous and synonymous mutations at a
235 broad level of the genome show several genes that may be under selective pressure.
236
237 Conclusion
238 The range of group variability of proteins was previously studied by various
239 groups of researchers on the example of the P17 proteins [34], P54 and CD2v [15, 26,
240 33].
241 Characteristics of the ASFV genes variability of the selected group are object of
242 great interest for searching for genetic differences and markers of evolutionary
243 variability in immunomodulatory proteins and can also serve to identify target sites in
244 the ASFV genome for vaccine development.
245 The results obtained from bioinformatics analysis showed that the 5EL and I14L
246 proteins have intracellular regions, the K11L protein has extracellular and intracellular
247 topological domains and a transmembrane region. The presence of single nucleotide
248 substitutions in these sites may indicate a greater conservatism of these proteins.
249 The degree of nucleotide substitutions of the concatenated *A238l, I329l* and
250 *Dp71l* ASFV genes, as determined in this study, revealed a purifying (stabilizing)
251 selection at the level of the nucleotide sequences. As a result of the nucleotide
252 sequences analysis obtained as a result of sequencing of the *A238l, I329l* and *Dp71l*
253 genes, it can be concluded that these genes are conservative, with variable regions
254 within them. All Russian ASFV isolates obtained in 2016-2017 have identical
255 sequences of the *A238l* and *I329l* genes, which indicate their common origin. The
256 topology of the phylogenetic tree according to the *I329l* gene completely corresponds
257 with the phylogenetic tree constructed on the basis of a pseudosequence of 7
258 immunomodulatory genes. Thus, the *I329l* gene may be a genetic marker of common

origin. ASFV I14L protein has two forms: a long one (184 amino acids) and a short one (from 70 to 72 amino acids), which is unique among the ASFV variants.

Using the data obtained, it is possible to predict the possible involvement of these genes in changing the level of virulence inherent in the selected strains, as well as the ability to be factors for the ASFV virulence.

The work was performed as part of the state assignment 0615-2017-0001.

Acknowledgments. This work was carried out within the framework of state task and was supported by the Ministry of Science and Higher Education of the Russian Federation.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval. This article does not contain any studies with human participants or animals as objects of research.

Contribution. Writing the text, collecting and processing the material, performing individual stages of the experimental part - Nefedeva M.V., gene sequencing - Titov I.A., statistical processing of protein variability - Mima K.A., conception and design of the study, text editing - Malogolovkin A.S.

REFERENCES

1. Sanchez-Vizcaino J.M. African Swine Fever. In: Straw B., D'Allaire S., Mengeling W., Taylor D., (eds). Diseases of Swine. 9th edition. USA: Iowa State University; 2006: 291-8.
2. Rodriguez J.M., Moreno L.T., Alejo A., Lacasta A., Rodriguez F., Salas M.L. Genome sequence of african swine fever virus BA71, the virulent parental strain of the nonpathogenic and tissue-culture adapted BA71V. *PLoS ONE*. 2015; 10(11): e0142889. DOI: 10.1371/journal.pone.0142889.
3. De Villiers E.P., Gallardo C., Arias M., Da Silva M., Upton C., Martin R. et al. Phylogenomic analysis of 11 complete African swine fever virus genome sequences. *Virology*. 2010; 400: 128-36. DOI: 10.1016/j.virol.2010.01.019.

286 4. Sanchez-Vizcaıno J. M., Mur L., Gomez-Villamandos J.C., Carrasco J.L. An
287 Update on the Epidemiology and Pathology of African Swine Fever. *J. Comp. Pathol.*
288 2015; 152: 9-21. DOI: 10.1016/j.jcpa.2014.09.003.
289 5. Rock D.L. Challenges for African swine fever vaccine development—"…
290 perhaps the end of the beginning." *Vet. Microbiol.* 2017; 206: 52-58. DOI:
291 10.1016/j.vetmic.2016.10.003.
292 6. Kolbasov D.V., Balyshev V.M., Sereda A.D. Results of research works on the
293 development of live vaccines against African swine fever. *Veterinariya*. 2014; 8: 3-8.
294 (in Russian)
295 7. King K., Chapman D., Argilaguet J.M., Fishbourne E., Hutet E., Cariolet R. et
296 al. Protection of European domestic pigs from virulent African isolates of African swine
297 fever virus by experimental immunisation. *Vaccine.* 2011; 29(28): 4593-600. DOI:
298 10.1159/000170936.
299 8. Dixon L.K., Abrams C.C., Chapman D.D., Goatley L.C., Netherton C.L., Taylor
300 G. et al. Prospects for development of African swine fever virus vaccines. *Dev Biol*
301 *(Basel)*. 2013; 135: 147-57. DOI: 10.1159/000170936.
302 9. Nix R.J., Gallardo C., Hutchings G., Blanco E., Dixon L.K. Molecular
303 epidemiology of African swine fever virus studied by analysis of four variable genome
304 regions. *Arch. Virol*. 2006; 151(12): 2475-94. DOI: 10.1007/s00705-006-0794-z.
305 10. Dixon L.K., Baylis S.A., Vydeingum S., Twigg S.R.F., Hammond J.M.,
306 Hingamp P.M. et al. African swine fever virus genome content and variability. *Arch.*
307 *Virol.* 1993; (7): 185-199. DOI: 10.1007/978-3-7091-9300-6_15.
308 11. Dixon L.K., Abrams C.C., Bowick G., Goatley L.C., Kay-Jackson P.C.,
309 Chapman D. et al. African swine fever virus proteins involved in evading host defence
310 systems. *Vet. Immunol. Immunopathol.* 2004; 100(3-4): 117-34.
311 DOI:10.1016/j.vetimm.2004.04.002
312 12. De Oliveira V.L., Almeida S.C., Soares H.R., Crespo A., Marshall-Clarke S.,
313 Parkhouse R.M.E. A novel TLR3 inhibitor encoded by African swine fever virus
314 (ASFV). *Arch. Virol.* 2011; 156: 597-609. DOI: 10.1007/s00705-010-0894-7.
315 13. Zsak L., Lu Z., Kutish G.F., Neilan J.G., Rock D.L. An african swine fever virus
316 virulence-associated gene NL-S with similarity to the herpes simplex virus ICP34.5
317 gene. *J. Virol.* 1996; 70(12): 8865-71.
318 14. Granja A.G., Perkins N.D., Revilla Y. A238L inhibits NF-ATc2, NF-kappa B,
319 and c-Jun activation through a novel mechanism involving protein kinase C-theta-

mediated up-regulation of the amino-terminal transactivation domain of p300. *J. Immunol.* 2008; 180(4): 2429-42. DOI: 10.4049/jimmunol.1490049

15. Chapman D.A., Tcherepanov V., Upton C., Dixon L.K. Comparison of the genome sequences of non-pathogenic and pathogenic African swine fever virus isolates. *J. Gen. Virol.* 2008; 89(2): 397-408. DOI: 10.1099/vir.0.83343-0

16. Garcia-Boronat M., Diez-Rivero C.M., Reinherz E.L., Reche P.A. PVS: a web server for protein sequence variability analysis tuned to facilitate conserved epitope discovery. *Nucleic Acids Res.* 2008; 36: 35-41. Doi:10.1093/nar/gkn211

17. Nielsen H. Predicting Secretory Proteins with SignalP. In: Kihara D., (eds). Protein Function Prediction. Springer, 2017: 59-73. Doi: 10.1007/978-1-4939-7015-5_6.

18. Dobson L., Remenyi I., Tusnady G. E. CCTOP: a Consensus Constrained TOPology prediction web server. *Nucleic Acids Res.* 2015; 43(1): 408-12. DOI: 10.1093/nar/gkv451.

19. Gallardo C., Mwaengo D.M., Macharia J.M., Arias M., Taracha E.A., Soler A. et al. Enhanced discrimination of African swine fever virus isolates through nucleotide sequencing of the p54, p72, and pB602L (CVR) genes. *Virus Genes.* 2009; 38(1): 85-95. DOI: 10.1007/s11262-008-0293-2.

20. Bastos A.D., Penrith M.L., Cruciere C., Edrich J.L., Hutchings G., Roger F. et al. Genotyping field strains of African swine fever virus by partial p72 gene characterisation. *Arch. Virol.* 2003; 148(4): 693-706. DOI: 10.1007/s00705-002-0946-8

21. Sereda A.D, Balyshev V.M. Antigenic diversity of African swine fever viruses. *Vopr Virusol*. 2011; 56(4): 38-42. (in Russian)

22. Edgar R.C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 2004; 32(5): 1792-7. DOI: 10.1093/nar/gkh340.

23. Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* 2013; 30(12): 2725-9. DOI: 10.1093/molbev/mst197.

24. Mosavi L.K., Cammett T.J., Desrosiers D.C., Peng Z.Y. The ankyrin repeat as molecular architecture for protein recognition. *Protein Sci.* 2004; 13(6): 1435-48. DOI: 10.1110/ps.03554604.

25. Jousse C., Oyadomari S., Novoa I., Lu P.D., Zhang Y., Harding H.P. et al. Inhibition of a constitutive translation initiation factor 2a phosphatase, CReP, promotes

353 survival of stressed cells. *J. Cell Biol.* 2003; 163: 767-775. DOI:
354 10.1083/jcb.200308075
355 26. Chapman D.A., Darby A.C., Da Silva M., Upton C., Radford A.D., Dixon L.K.
356 Genomic analysis of highly virulent Georgia 2007/1 isolate of African swine fever
357 virus. *Emerging Infect. Dis.* 2011; 17(4): 599-605. DOI: 10.3201/eid1704.101283.
358 27. Neilan J.G., Lu Z., Kutish G.F., Zsak L., Lewis T.L., Rock D.L. A conserved
359 African swine fever virus I kappa B homolog, 5el, is nonessential for growth in vitro
360 and virulence in domestic swine. *Virology.* 1997; 235(2): 377-85.
361 DOI:10.1006/viro.1997.8693.
362 28. Abrams C.C., Chapman D.A., Silk R., Liverani E., Dixon L.K. Domains
363 involved in calcineurin phosphatase inhibition and nuclear localisation in the African
364 swine fever virus A238L protein. *Virology.* 2008; 374(2): 477-86. DOI:
365 10.1371/journal.pbio.1001492.
366 29. Mima K.A., Burmakina G.S., Titov I.A., Malogolovkin A.S. African swine fever
367 virus glycoproteins p54 and CD2v in the context of immune response modulation:
368 bioinformatic analysis of genetic variability and heterogeneity. Sel'skokhozyaistvennaya
369 biologiya. 2015; 50(6): 785-93. doi: 10.15389/agrobiology.2015.6.785rus. (in Russian)
370 30. Bishop R.P., Fleischauer C., de Villiers E.P., Okoth E.A., Arias M., Gallardo C.
371 et al. Comparative analysis of the complete genome sequences of Kenyan African swine
372 fever virus isolates within p72 genotypes IX and X. *Virus Genes.* 2015; 50(2): 303-9.
373 DOI: 10.1007/s11262-014-1156-7.
374 31. Brown J.R., Douady C.J., Italia M.J., Marshall W.E., Stanhope M.J. Universal
375 trees based on large combined protein sequence data sets. *Nat .Genet.* 2001; 28: 281-
376 285. DOI: 10.1038/90129.
377 32. Miya M., Nishida M. Use of mitogenomic information in teleostean molecular
378 phylogenetics: a tree-based exploration under the maximum-parsimony optimality
379 criterion. *Mol. Phylogenet. Evol*. 2000; 17: 437-55. DOI:10.1006/mpev.2000.0839.
380 33. Rowlands R.J., Duarte M.M., Boinas F., Hutchings G., Dixon L.K. The CD2v
381 protein enhances African swine fever virus replication in the tick vector, Ornithodoros
382 erraticus. *Virology.* 2009; 393(2): 319-28. DOI: 10.1016/j.virol.2009.07.040.
383 34. Suarez C., Gutierrez-Berzal J., Andres G., Salas M.L., Rodriguez J.M. African
384 Swine Fever Virus Protein p17 Is Essential for the Progression of Viral Membrane
385 Precursors toward Icosahedral Intermediates. *J. Virol.* 2010; 84(15): 7484-99. DOI:
386 10.1128/JVI.00600-10.

387

388

389 **Fig.1. The results of the variability analysis of proteins 5EL - IkB homolog and**

390 **inhibitor calceneurin phosphatase; I14L - a protein similar to HSV neurovirulence**

391 **factor ICP34.5; K11L - a TLR signaling inhibitor protein.**

392 **Fig.2. Phylogenetic dendrogram constructed by the maximum likelihood method**

393 **based on concatenated nucleotide sequences of ASFV 15 strains of 7**

394 **seroimmunotypes. The legend indicates the strains and isolates: - isolated**

395 **from pigs, - ticks, - warthogs and - adapted to cell culture.**

396 **Fig.3. Electrophoregram of the amplification products of the *Dp71l* gene encoding**

397 **I14L protein. M = 100 b.p.**

398

399 **Table.**

400 **ASFV strains used in the work**

401

402

403

404

405

406

407

408

409

410

411

412

413

414

Strain/isolate	Country	Isolated year	Genotype	Seroimmunotype	A238l	I329l	Dp71l
Lisbon-57 (L-57)	Portugal	1957	I	1	MG209277	MG209292	MF589625
LK-111	Portugal	1978	I	1	MG209278	MG209293	MF589627
Kongo-49 (K-49)	Zaire	1949	I	2	MG209275	MG209290	MF589624
KK-262	Zaire	-	I	2	MG209276	MG209291	MF589626
France-32 (F-32)	France	1964	I	4	MG209272	MG209288	MF589621
FK-32/135	France	Passaged from F-32	I	4	MG209273	MG209289	MF589622
CKA-2015	Russia	Passaged from Stavropol 01/08	I I	8	MG010372	MG209287	MF589620
Irkutsk-2017	Russia	2017	I I	8	MG209274	-	MF589623
Mozambique-78 (M-78)	Mozambique	1978	V	3	MG209279	MG209294	MF589628
MK-200	Mozambique	Passaged from M-78	V	3	MG209280	MG209295	MF589630
TKF	Tanzania	-	X	3	MG209282	MG209297	MF589631
TSP-80	Tanzania	Passaged in 1980 г.	X	5	MG209285	MG209300	MF589634
TSP-80/300	Tanzania	-	X	5	MG209286	MG209301	MF589635
TS-7	Tanzania	Passaged in 1984 г.	X	6	MG209283	MG209298	MF589632
TS-7/27-230	Tanzania	Passaged in 1986 г.	X	6	MG209284	MG209299	MF589633
Nanyuki	Kenya	1960		8	MG209281	MG209296	MF589629
Lipetsk-2016 Tambov-2016 Moscow-2016 Penza-2016 Pskov-2016 Voronezh-2016 Arkhangelsk-2016 Kursk-2016 Krasnodar-2016	Russia	2016	I I	8	-	-	-

415