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Analysis of the whole-genome sequence of an ASF virus (*Asfarviridae: Asfivirus: African swine fever virus*) isolated from a wild boar (*Sus scrofa*) at the border between Russian Federation and Mongolia

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Introduction. The causative agent of African swine fever (*Asfarviridae: Asfivirus: African swine fever virus*) (ASF) is a double-stranded DNA virus of 175–215 nm. To date, 24 of its genotypes are known. Clustering of ASF genotype II isolates is carried out by examining a limited number of selected genome markers. Despite the relatively high rate of mutations in the genome of this infectious agent compared to other DNA viruses, the number of known genome molecular markers for genotype II isolates is still insufficient for detailed subclustering.
The **aims** of this work were the comparative analysis of ASFV/Zabaykali/WB-5314/2020 virus isolate and determination of additional molecular markers which can be used for clustering of viral genotype II sequences.
Material and methods. ASF virus isolate ASFV/Zabaykali/WB-5314/2020 was used to extract genomic DNA (gDNA). Sequencing libraries were constructed using the Nextera XT DNA library prepare kit (Illumina, USA) using the methodology of the next generation sequencing (NGS).
Results. The genome length was 189,380 bp, and the number of open reading frames (ORFs) was 189. In comparison with the genome of reference isolate Georgia 2007/1, 33 single nucleotide polymorphisms (SNPs) were identified, of which 13 were localized in the intergenic region, 10 resulted to the changes in the amino acid sequences of the encoded proteins, and 10 affected the ORF of ASF virus genes.
Discussion. When analyzing intergenic regions, the ASFV/Zabaykali/WB-5314/2020 isolate is grouped separately from a number of isolates from Poland and three isolates from People's Republic of China (PRC), since it does not harbor additional tandem repeat sequence (TRS). At the same time, the construction of a phylogenetic tree based on *DP60R* gene sequencing relates ASFV/Zabaykali/WB-5314/2020 to isolates from PRC and Poland. Moreover, phylogenetic analysis of full-genome sequences confirmed previous studies on the grouping of viruses of genotype II, and as for the studied isolate, it was grouped with the variants from China.
Conclusion. A new variable region was identified, the *DP60R* gene, clustering for which gave a result similar to the analysis of full-length genomes. Probably, further study of the distribution of ASF virus isolates by groups based on the analysis of this gene sequences will reveal its significance for studying the evolution of the virus and its spread.

Key words: *African swine fever virus, full genome sequencing, genetic analysis, phylogenetic analysis*

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НАУЧНАЯ СТАТЬЯ
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Анализ полногеномной последовательности изолята вируса африканской чумы свиней (*Asfarviridae: Asfivirus: African swine fever virus*), выделенного от дикого кабана (*Sus scrofa*) на границе Российской Федерации и Монголии

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Введение. Возбудитель африканской чумы свиней (*Asfarviridae: Asfivirus: African swine fever virus*) (АЧС) – двухцепочечный ДНК-вирус размерами 175–215 нм. На сегодняшний день известно 24 его генотипа. Кластеризация вирусов АЧС II генотипа проводится путём изучения ограниченного числа выбранных областей генома. Несмотря на относительно высокую скорость накопления замен в геноме этого инфекционного агента по сравнению с другими ДНК-содержащими вирусами, число известных геномных молекулярных маркёров для изолятов II генотипа до настоящего времени недостаточно для детальной субкластеризации.
Целями данной работы являлись сравнительный анализ изолята вируса АЧС ASFV/Zabaykali/WB-5314/2020 и определение дополнительных молекулярных маркёров, использование которых возможно при кластеризации вируса II генотипа.
Материал и методы. В работе использован изолят вируса АЧС ASFV/Zabaykali/WB-5314/2020. Библиотеку последовательностей конструировали с использованием набора Nextera XT DNA library preparation kit (Illumina, США) с помощью методики секвенирования нового поколения (next generation sequencing, NGS).
Результаты. Длина генома исследуемого изолята составила 189 380 п.н., число открытых рамок считывания (ОРС) – 189. При сравнении с референтным геномом Georgia 2007/1 у варианта обнаружены 33 однонуклеотидных полиморфизма (ОНП). Из них 13 локализуются в межгенных областей 10 приводят к изменению аминокислотных последовательностей кодируемых белков и 10 – оказывают влияние на ОРС генов вируса АЧС.
Обсуждение. По данным анализа межгенных областей ASFV/Zabaykali/WB-5314/2020 группируется отдельно от нескольких изолятов из Польши и 3 – из Китайской Народной Республики (КНР), поскольку не содержит тандемных повторов (tandem repeat sequences, TRS). В то же время при построении филогенетического дерева на основании секвенирования гена *DP60R* данный изолят оказался объединённым с таковыми из КНР и Польши. При этом филогенетический анализ полногеномных последовательностей подтвердил результаты предшествующих исследований по кластеризации вирусов АЧС II генотипа, а вариант ASFV/Zabaykali/WB-5314/2020 оказался распределённым в группу с изолятами из Китая.
Заключение. Идентифицирована новая вариабельная область генома вируса АЧС – ген *DP60R*, кластеризация по которому дала результат, аналогичный таковому при анализе полноразмерных геномов. Можно предполагать, что дальнейшие работы по групповому распределению изолятов вируса АЧС, базирующиеся на изучении последовательности этого гена, позволят выявить значимость данной генной структуры для научных изысканий в отношении эволюции инфекционного агента и путей его распространения.

Ключевые слова: *африканская чума свиней (АЧС), полногеномное секвенирование, генетический анализ, филогенетический анализ*

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Introduction

African swine fever (ASF) is a highly deadly hemorrhagic disease affecting domestic and wild pigs (*Suidae*). In 2020, Russia reported 271 ASF outbreaks, including 161 outbreaks among domestic pigs (*Sus scrofa domesticus*) and 110 outbreaks in the population of wild boars (*Sus scrofa*) [17].

The pathogen is a DNA-containing arbovirus comprising an icosahedral capsid and supercapsid envelope. Its genome is a linear double-stranded DNA molecule with covalently closed ends and inverted terminal repeats (ITR) [14]. The central conserved region of the genome is ~125 kbp in length, which varies within 1.5% (depending on the isolate) as opposed to 2 highly variable regions located close to the DNA ends [5, 6]. Based on the analysis of the sequence of the C-terminal region of the highly conserved *B646L* gene encoding the major capsid protein p72, all the known African swine fever virus (ASFV) isolates and strains can be classified into 24 genotypes [1, 3].

At the same time, the central conserved region has localized zones of high variability, for example, the CVR-region of the *B602L* gene, which is caused by changes in the number of tandem repeat sequences (TRS). In some cases, their number can be used for identification of virus relatedness and for differentiation among closely related variants [9].

The genomic analysis of the isolates collected in ASF affected countries from 2007 to 2020 is based on the analysis of the single *B646L* gene and does not make it possible to identify routes of importation and transmission of the virus in different areas, as it clusters all variants of genotype II. The isolates can be differentiated only through the analysis of additional marker regions of the ASFV, for example, through identification of intergenic regions and the number of tandem repeats in them.

Following the identification of an insertion of 1 or 2 TRS of 10 base pairs in the intergenic region (IGR) between *I73R* and *I329L*, we were able to divide isolates of genotype II into 4 groups [9]. The ASF reference laboratory at the FGBI «Federal Centre for Animal Health» («ARRIAH») identified an additional 17-nucleotide TRS-insertion in the intergenic region *MGF505-9R*/*MGF505-10R* (MGF-2). In its turn, the analysis of this region made it possible to identify 3 additional clusters among the genotype II isolates [8].

The analysis of commonly selected genome fragments (such as *B602L*, *B646L*, or *E183L*) in the variants belonging to ASFV genotype II does not provide sufficient information for phylogenetic clustering of closely related isolates. On the other hand, whole-genome sequencing offers the possibility to identify both the known and new genomic markers. The wide array of data on the identified variable regions of the viral genome turns the whole-genome sequencing of the isolates detected in Russia and in other countries into the only method that gives additional information about disease epizootiology. Taken together, all these data provide an efficient tool for tracking the transmission paths of the infectious agent.

The aim of this study was to perform a comparative analysis of the ASFV/Zabaykali/WB-5314/2020 isolate and to identify additional molecular markers to be further used in clustering the genotype II ASF virus.

We report the results of the whole-genome sequencing and comparative studies of the genome of the virus isolate obtained from a wild boar and identified as a causative agent of the first infection outbreak in southeast Russia (the Trans-Baikal (Zabaikalie) Territory) in close proximity to the border with Mongolia.

Material and methods

Virus isolates. In our tests, we used a sample of the pathological material from a wild boar found dead in Menza rural settlement in the Krasnochikoysky District in the Trans-Baikal Territory of the Russian Federation

(geographic coordinates 49.364383, 108.549859), ~2.5 km from the border with the People's Republic of China (PRC). The sample was studied using real-time polymerase chain reaction (real-time PCR) and virus isolation methods, which helped us detect the ASF causative agent and its genome in the sample [2]. This isolate was named ASFV/Zabaykali/WB-5314/2020.

Culturing, titration, and purification of the virus. To accumulate the virus, we used a porcine spleen cell culture (PSC) [11, 13]. The accumulated virus-containing material was combined and used to extract genomic DNA (gDNA).

Genomic DNA extraction. gDNA was extracted by using the phenol-chloroform method; the resulting residue was eluted in nuclease-free water [7].

Whole-genome sequencing, sequence assembly and alignment. The sequencing library was constructed using the Nextera XT DNA library preparation kit (Illumina, United States). The next generation sequencing (NGS) was performed using the MiSeq reagent kit version 2 (Illumina) and 250 cycles of paired-end tag reading with a high-throughput MiSeq ILLUMINA sequencing system (Illumina). The assembled genome sequence was aligned against the reference genome of variant FR682468.1_ASFV/Georgia 2007/1. The average depth of coverage was ×45, and the fragment length 250 bp.

Open reading frames (ORFs) were identified with the GATU software. The complete genomic sequence was deposited in GenBank under accession number MZ325862.

Phylogenetic analysis. Prior to the comparative genetic analysis, we collected data on the previously sequenced ASFV isolates from Russia and neighboring countries from GenBank **(Table 1**). The above sequences were used for detection of single nucleotide polymorphisms (SNPs), alignment and identification of phylogenetic relatedness of the isolates. Construction, alignment and identification of SNPs were completed with the CLC Genomics Workbench v.9 software (QIAGEN, Aarhus, Denmark; www.clcbio.com).

The phylogenetic analysis of sequences was performed by the maximum likelihood (ML) method and comparison with 1,000 bootstrap iterations using the general time-reversible (GTR) model, GTR + G + I (general time reversible + gamma distribution + independent rates) = 4, and the Mega X software [12, 14].

The statistical analysis was conducted using the STATISTICA 10.0 software package to calculate the mean value (*M*), and the standard deviation (*SD*). Differences were considered statistically significant at $p \leq 0.001$.

Results

Virus isolation and culturing. The hemadsorption in the PSC culture inoculated with the virus-containing suspension made from wild boar organs occurred on the 3rd day of incubation at 37° C. Subsequently, the virus-containing suspension was used in the serial passaging of the pathogen in the above cell culture.

The virus titer was expressed as the highest dilution causing hemadsorption in 50% of the infected cell cultures, per 0.1 ml, and expressed as log 10 (lg) of the hemadsorption unit (HAU). The titer levels were calculated using the guidelines developed by Mazloum A. et al. [13].

The analysis of changes in the virus titer levels showed a steady increase in the pathogen accumulation with each subsequent passage (**Table 2**), thus indicating that the virus successfully replicated in the cell culture without demonstrating any changes in its hemadsorption activity. These findings were of high importance for the further virus accumulation and purification from the large amount of the virus-containing suspension.

The Illumina sequencing was used to identify the complete genomic sequence of genome ASFV/Zabaykali/WB-5314/2020, which was 189,380 bp long. The GATU software was used to detect the presence of 189 ORFs in this genome. The whole-genome sequence was thoroughly studied, and it was found that the above isolate belonged to genotype II [1].

Fig. 1 shows the phylogenetic tree built using the results of the analysis of the C-terminal fragment of the B646L gene of virus isolate ASFV/Zabaykali/WB-5314/2020.

As can be seen in **Fig. 1**, all ASFV isolates collected in Europe and Asia during 2007–2020, including ASFV/Zabaykali/WB-5314/2020, belong to genotype II. Based on the analysis of the intergenic regions (*I73R*/*I329L*) and *MGF505* (*9R-10R*), the above variant was assigned to cluster IGR-1 (**Fig. 2**) and subgroup MGF-1 [8].

It can be seen that ASFV/Zabaykali/WB-5314/2020 belongs to group IGR-1, together with variants Georgia 2007/1 (Georgia), China ASFV-wbBS01 (PRC), the isolates from Russia (ASFV/Amur 19/WB-6905 and ASFV/Kabardino-Balkaria 19/WB-964) and Poland (Pol17 03029 C201 and Pol16 29413 o23). However, other isolates from the same countries and from Czech Republic, Moldova, and Belgium had an insertion in the above intergenic region and, consequently, belonged to cluster IGR-2.

The virus variants clustered solely by comparison of the 2 described genome regions failed to show any consistency with the results of the phylogenetic analysis of whole-genome sequences. The comparison of genome sequences of variant ASFV/Zabaykali/WB-5314/2020 with 15 samples received from PRC led to the conclusion that all the studied isolates should be grouped into individual cluster East. The respective phylogenetic tree is shown in **Fig. 3**.

SNPs were identified using the data resulting from the alignment against reference isolate Georgia 2007/1. The analysis resulted in detection of 33 SNPs with 13 of them being localized in intergenic regions; 10 of them cause changes in the amino acid sequence of the proteins encoded by them; and the other 10 have an effect on ORFs of ASFV genes. More detailed information about the location of SNPs is given in **Table 3**.

The *DP60R* gene was used as a marker for clustering ASFV isolates in accordance with the recommendations provided by Wen X. et al. [18]. As a result, the analyzed isolates were divided into 3 groups [18] (**Fig. 4**). As can be seen in **Fig. 4**, the ASFV isolates were clustered by the following principle: group I included isolates having

Table 1. Information about ASF virus isolates retrieved from GenBank database
Таблица 1. Сведения об использованных изолятах вируса АЧС из базы GenBank

Isolate number Номер изолята	Isolate name Название изолята	Country Страна	GenBank accession number Номер доступа в GenBank
1	Georgia 2007/1	Georgia Грузия	FR682468.2
2	ASFV/Primorsky 19/WB-6723	Russia Россия	MW306190
3	ASFV/Amur 19/WB-6905	Russia Россия	MW306191
4	ASFV/Ulyanovsk 19/WB-5699	Russia Россия	MW306192
5	ASFV/Kabardino-Balkaria 19/WB-964	Russia Россия	MT459800
6	Odintsovo 02/14	Russia Россия	KP843857.1
7	ASFV/LT14/1490	Latvia Латвия	MK628478.1
8	China Pig/HLJ/2018	China Китай	MK333180.1
9	China/2018/AnhuiXCGQ	China Китай	MK128995.1
10	China ASFV-wbBS01	Китай China	MK645909.1
11	China DB/LN/2018	China Китай	MK333181.1
12	Pol17_04461_C210	Poland Польша	MG939588.1
13	Pol17_03029_C201	Poland Польша	MG939587.1
14	Pol16_29413_o23	Poland Польша	MG939586.1
15	Pol16_20540_o10	Poland Польша	MG939585.1
16	CzechRepublic 2017/1	Czech Republic Чехия	LR722600.1
17	Moldova 2017/1	Moldova Молдова	LR722599.1
18	Belgium 2018/1	Belgium Бельгия	LR536725.1

Table 2. Indicators of accumulation titer of African swine fever virus isolate ASFV/Zabaykali/WB-5314/2020 (n = 3) in the cell culture
Таблица 2. Показатели титра накопления изолята вируса африканской чумы свиней ASFV/Zabaykali/WB-5314/2020 (n = 3) в культуре клеток

Virus isolate Изолят вируса		Virus titer in the passage, lg HAU_{50} ($M \pm SD$) Титр вируса в пассаже, lg $ГАдЕ_{50}$ ($M \pm SD$)		
	Passage number Номер пассажа	1	2	3
ASFV/Zabaykali/WB-5314/2020		4,21 ± 0,26	5,66 ± 0,14	6,02 ± 0,12

Note. HADU, hemadsorption unit.

Примечание. ГАдЕ – гемадсорбирующая единица.

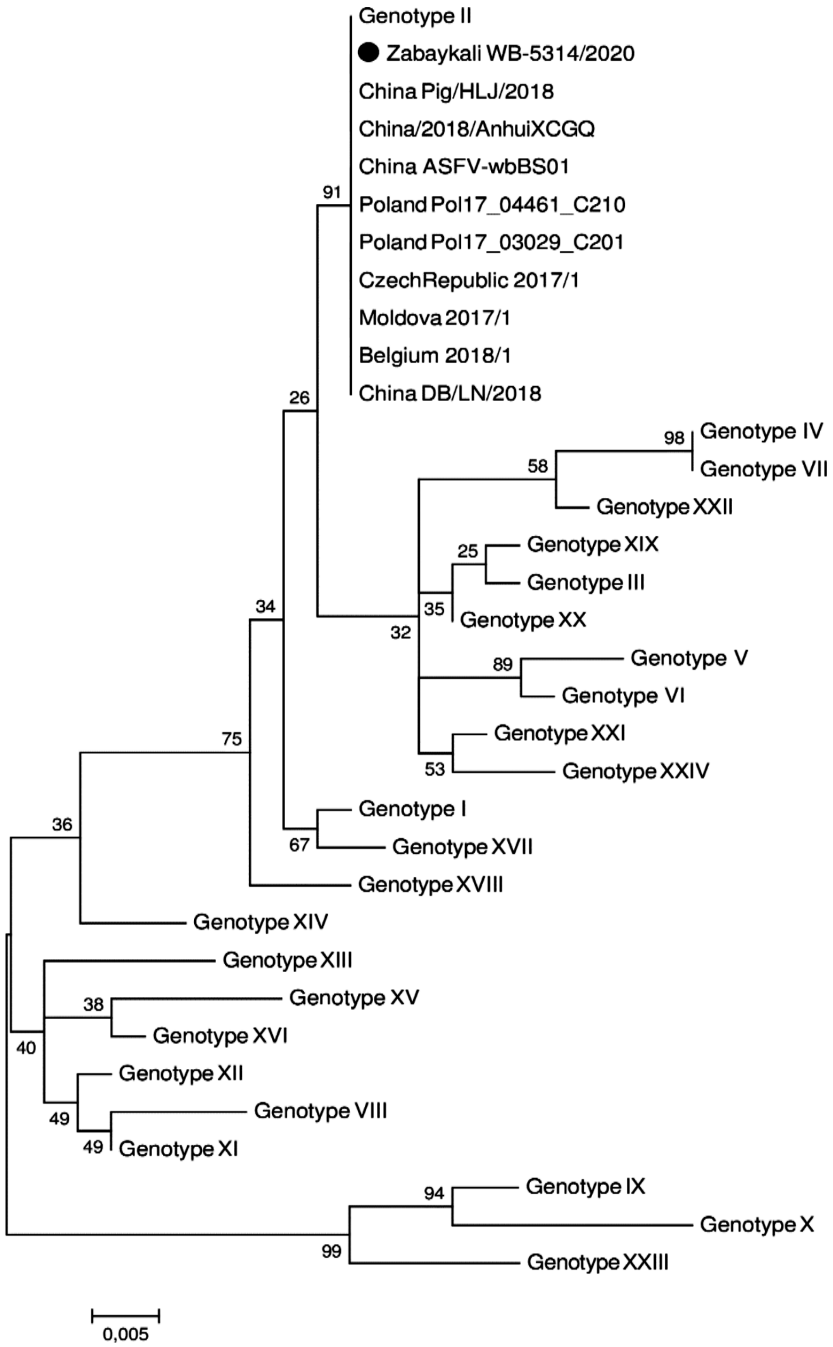

Fig. 1. Phylogenetic tree based on the analysis of the C-terminal fragment of the *B646L* gene of ASFV/Zabaykali/WB-5314/2020 isolate of the African swine fever virus.

Note. Isolate ASFV/Zabaykali/WB-5314/2020 is indicated with black circle.

Рис. 1. Филогенетическое дерево, построенное на основании данных анализа С-концевого фрагмента гена *B646L* изолята ASFV/Zabaykali/WB-5314/2020 вируса африканской чумы свиней.

Примечание. Изолят ASFV/Zabaykali/WB-5314/2020 обозначен кружком черного цвета.

the same amino acid composition as the reference isolate Georgia 2007/1; group II combined isolates having a substitution of isoleucine (Ile, I) for asparagine (Asp, N) at position 38. Finally, group III was represented by the only isolate ASFV/LT14/1490, which has a substitution of lysine (Lys, K) with isoleucine (Ile, I) at position 9. The findings of the analysis of this region show that variant ASFV/Zabaykali/WB-5314/2020 is related to 3 isolates from PRC, 2 from Russia, and 4 from Poland (**Fig. 4**).

We also performed a phylogenetic analysis of the combined amino acid sequences of the *MGF505-9R*, *C84L*, *I267L,* and *DP60R* genes [16, 18] (**Fig. 5**). Based on the

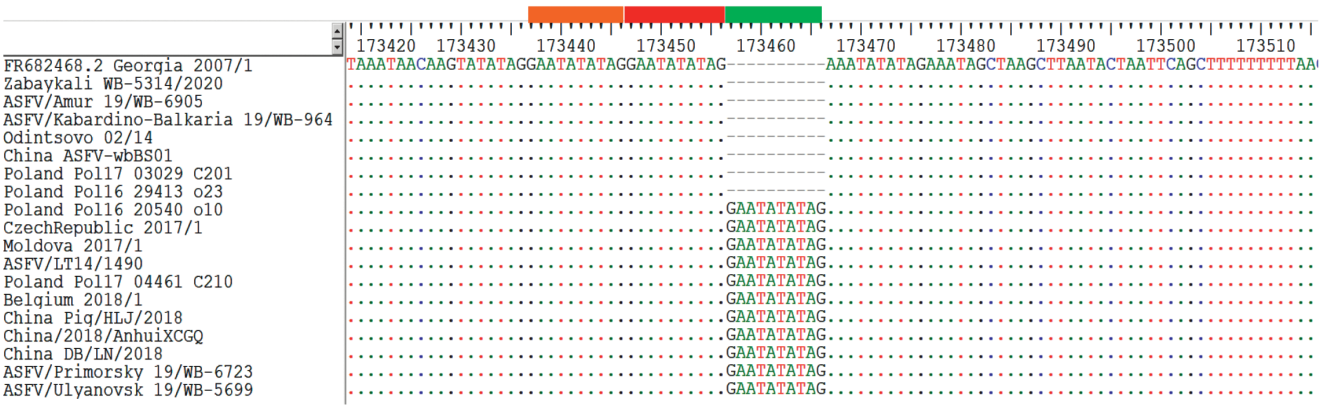

Fig. 2. Nucleotide sequences of the *I73R*/*I329L* intergenic region of the African swine fever virus isolates used in the study.

Note. Each letter represents one nucleotide according to international nomenclature. Tandem repeats present in the genome are marked in orange and red; an additional tandem repeat is highlighted in green.

Рис. 2. Нуклеотидные последовательности межгенной области *I73R*/*I329L* изолятов вируса африканской чумы свиней, использованных в исследовании.

Примечание. Каждая буква представляет один нуклеотид в соответствии с международной номенклатурой. Присутствующие в геноме тандемные повторы обозначены оранжевым и красным цветами, дополнительный тандемный повтор выделен зелёным цветом.

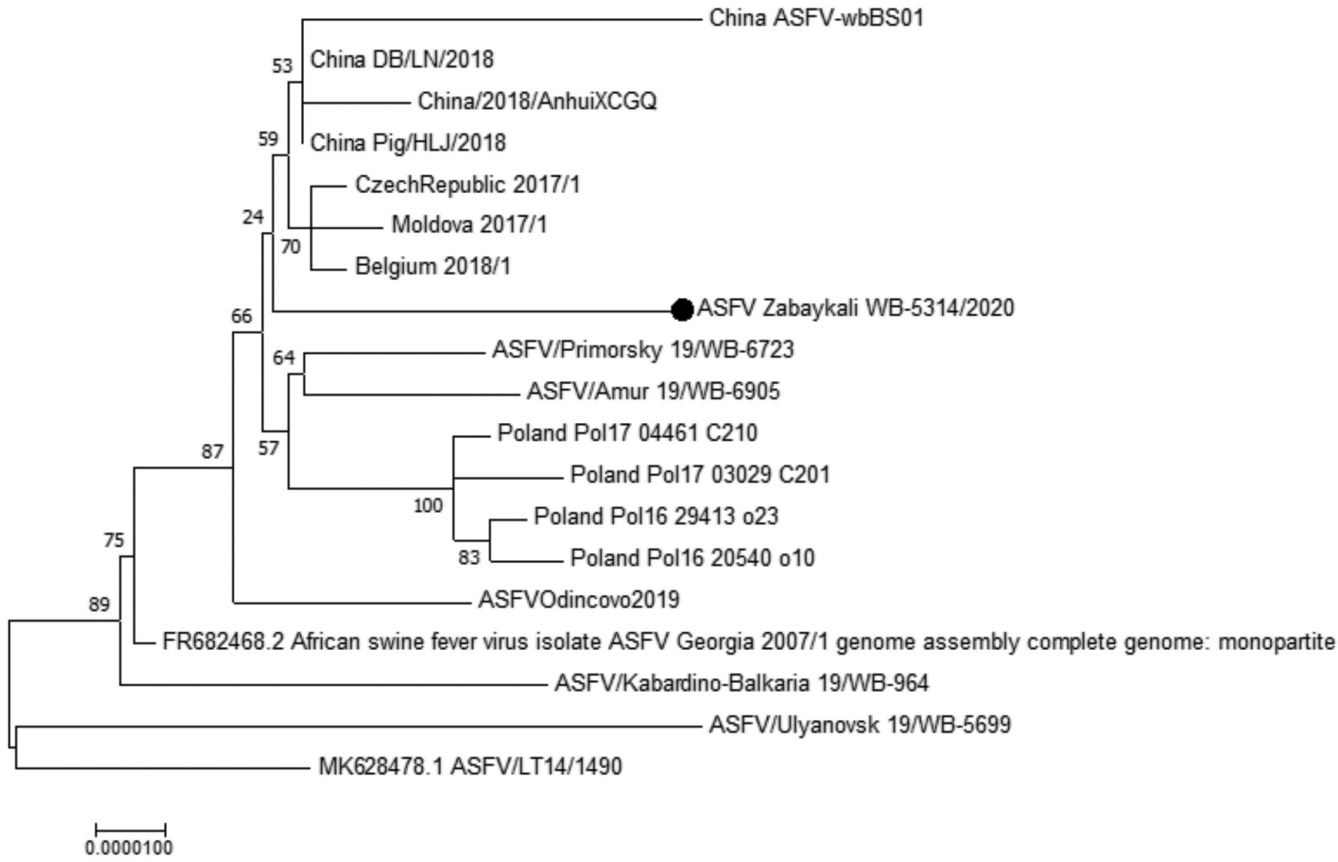

Fig. 3. Phylogenetic tree based on the analysis of 19 complete genome sequences of the African swine fever virus belonging to genotype II.

Note. Isolate obtained from the Russian Federation is indicated with black circle.

Рис. 3. Филогенетическое дерево, построенное на основании данных анализа 19 полных геномных последовательностей вируса африканской чумы свиней II генотипа.

Примечание. Изолят, выделенный на территории Российской Федерации, обозначен кружком черного цвета.

Table 3. Predicted single-nucleotide polymorphisms in the ASFV/Zabaykali/WB-5314/2020 genome compared with the Georgia/2007-1 variant and other isolates from the Russian Federation, their significance or effect on the proteins' amino acid sequences

Таблица 3. Предсказуемые однонуклеотидные полиморфизмы в геноме ASFV/Zabaykali/WB-5314/2020 по сравнению с вариантом Georgia/2007-1 и другими изолятами из Российской Федерации, их значение или влияние на аминокислотные последовательности белков

Gene Ген	Position (amino acid, substitution) Позиция (аминокислота, замена)	ASFV/Primorsky 19/WB-6723	ASFV/Ulyanovsk 19/WB-5699	ASFV/Amur 19/WB-6905	ASFV/Kabardino-Balkaria 19/WB-964	Georgia/2007-1 (FR682468.2)	ASFV/ Zabaikaly
Non-synonymous single-nucleotide polymorphisms Несинонимичные однонуклеотидные полиморфизмы							
KP177R	77: V→Y	V	V	V	V	V	Y
MGF360-10L	329: N→S	**S**	N	**S**	N	N	**S**
MGF505-4R	253: H→Y	H	H	H	H	H	Y
MGF505-9R	323: E→K	E	**K**	E	**K**	**K**	E
A137R	92: P→S	S	S	S	S	S	P
*C84L**	28: R→K	R	R	R	R	R	K
NP419L	414: S→N	S	**N**	S	**N**	**N**	S
E199L	85: A→P	A	A	A	A	A	P
*I267L**	235: Q→K	Q	Q	Q	Q	Q	K
I267L	195: F→I	F	**I**	F	**I**	**I**	F
Intergenic region Межгенная область							
Повторяющаяся область *KP93L* Repeat region *KP93L*	Point mutation (substitution or deletion) Точечная мутация (замена или делеция)	T	T	T	T	T	A
		T	T	T	T	T	A
		C	C	C	C	C	T
		–	–	–	–	–	A
MGF360-3L / MGF110-L		–	T	–	T	T	–
ASFV-G-ACD_00320* / ASFV-G-ACD_00330*	Homopolymer Гомополимер	7G	7G	7G	7G	8G	7G
MGF300-1L / MGF300-2R**		13G	12G	12G	11G	9G	11G
MGF360-10L / MGF360-11L**		10T	10T	10T	10T	10T	10T
MGF360-10L / MGF360-11L**		5A	5A	5A	5A	5A	4A
B602L / B385R**		7G	6G	7G	6G	6G	7G
CP123L / CP2475L	Point mutation (substitution or deletion) Точечная мутация (замена или делеция)	A	A	A	A	A	–

See p. 9 for continuation of Tab. 3. 161.

Continued Tab. 3 from p. 161.

Gene Ген	Position (amino acid, substitution) Позиция (аминокислота, замена)	ASFV/Primorsky 19/WB-6723	ASFV/Ulyanovsk 19/WB-5699	ASFV/Amur 19/WB-6905	ASFV/Kabardino-Balkaria 19/WB-964	Georgia/2007-1 (FR682468.2)	ASFV/ Zabaikaly
I73R / I329L	Tandem repeat sequence Тандемная повторяющаяся последовательность	IGR-2	IGR-2	IGR-1	IGR-1	IGR-1	IGR-1
ASFV-G-ACD_01990 / *DP60R*	Point mutation (substitution or deletion) Точечная мутация (замена или делеция)	A	A	A	A	A	T
	Alternative open reading frames Альтернативные открытые рамки считывания						
MGF110-1L	196 vs 214 aa/a.o.	T – 196	C – 214	C – 214	T – 196	C – 214	T – 196
ASFV-G-ACD_00190	(40 → 47) A → –	47 (–)	47 (–)	47 (–)	47 (–)	40 (A)	47 (–)
MGF110-11L	Homopolymer C* Гомополимер C*	9C	11C	13C	13C	13C	11C
MGF110-13Lb	A → C (G = G)	A	A	A	A	A	C-terminate in 6 aa С-конец с 6 амино-кислоты
MGF110-13Lb	Homopolymer C* Гомополимер C*	11C	13C	9C	10C	17C	15C
ASFV-G-ACD_00290	Homopolymer G* Гомополимер C*	8G	12G	8G	12G	10G	8G
MGF300-2R	76: N (160 aa/a.o.) → E + C-terminate (С-конец) (76 aa/a.o.)*	N (160)	N (160)	N (160)	N (160)	N (160)	E (76)
C84L	23: Q → K (G → –)	K	K	K	K	K	Q
C717R	592: E → R (G → –)	E	E	E	E	E	R Terminate early (ран-ний конец)
DP60R	38: N → I (A → –)	N	I	N	I	I	N

Note. The * after the ORF name indicates that the SNP is part of a homopolymer. For homopolymers, the number of repeated nucleotides is indicated (e.g., 7G means that there are 7 guanine residues in the series). If the SNP causes early termination of a protein (e.g., *MGF300-2R*), then one protein is predicted to consist of 76 amino acids and the others are predicted to consist of 160 amino acids).

Примечание. Знак * после названия ОРС указывает на то, что ОНП является частью гомополимера. Для гомополимеров указанно количество повторяющихся нуклеотидов (например, 7G означает, что в ряду присутствует 7 остатков гуанина). Если ОНП вызывает раннюю терминацию белка (например, *MGF300-2R*), то прогнозируется, что один белок состоит из 76 амино-кислот, а остальные – из 160 аминокислот).

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results of the analysis, isolates from PRC and Europe were clustered with isolates ASFV/Primorsky 19/WB-6723 and ASFV/Amur 19/WB-6905 from Russia. At the same time, they differed from isolates ASFV/Ulyanovsk 19/WB-5699 and ASFV/Kabardino-Balkaria 19/WB-964 collected in Russia in 2019. ASFV/Zabaykali/WB-5314/2020 did not belong to the group represented by the above variants.

Discussion

During 11 years in Russia, ASF outbreaks were recorded only in its western regions. However, in 2017, several outbreaks among domestic pigs were reported by the Irkutsk and Omsk Regions in Southeastern Siberia.

By the end of 2018 and at the beginning of 2019, several ASF outbreaks were recorded in PRC. In the same year, they were followed by similar outbreaks in the Far East in Russia, in the areas bordering PRC. Based on the data from Wen X. et al., [18], the genetic analysis found that ASFV isolates from PRC showed the highest similarity with the variants isolated in Poland, Belgium, Moldova, and Czech Republic in 2015–2018 [18].

Genotype II ASF viruses are clustered through the analysis of the limited number of the selected genomic regions (intergenic regions between *I73R* and *I329L*): *C84L*, *MGF360-1L*, *I267L*, *DP60R*, and *MGF360-16R* [9, 10, 18]. Although substitutions in the genome of this virus are accumulated at a relatively high rate compared to other DNA-containing viruses (from 10^{-4} to 10^{-5} substitutions/site/year), the number of the known genomic molecular markers for genotype II isolates is still too limited to complete detailed sub-clustering [3].

Today, the whole-genome sequencing method remains the preferred choice for identification of phylogenetic relatedness and for analysis of ASFV distribution in different geographic regions. As mutations in the ASFV genome are characterized by higher levels compared to other DNA viruses, the degree of relatedness among isolates by using a single (separately selected) genetic marker cannot be identified, while using different combinations of markers for a phylogenetic analysis can result in the situation when the same isolates are grouped into different clusters. Based on the analysis of the intergenic regions (**Fig. 2**), variant ASFV/Zabaykali/WB-5314/2020 is clustered separately from several isolates from Poland and 3 isolates from PRC, as it does not contain TRS. At the same time, the phylogenetic tree based on the sequencing of the *DP60R* gene (**Fig. 4**) combines ASFV/Zabaykali/WB-5314/2020 with isolates from PRC and Poland.

It should be noted that clustering of ASFV isolates by using the limited number of marker regions of the genome is least time-consuming, but is notable for varying clustering results when different combinations of markers are applied. Furthermore, any mutation outside the limits of the regions selected for the analysis will be neglected, though it is responsible for changes in the biological properties of the virus. Yet, the phylogenetic analysis of whole-genome sequences is consistent with the findings of the previous studies in clustering genotype II viruses, and the isolate, which was obtained in Russia, is assigned to the group that includes isolates from PRC [18] (**Fig. 3**).

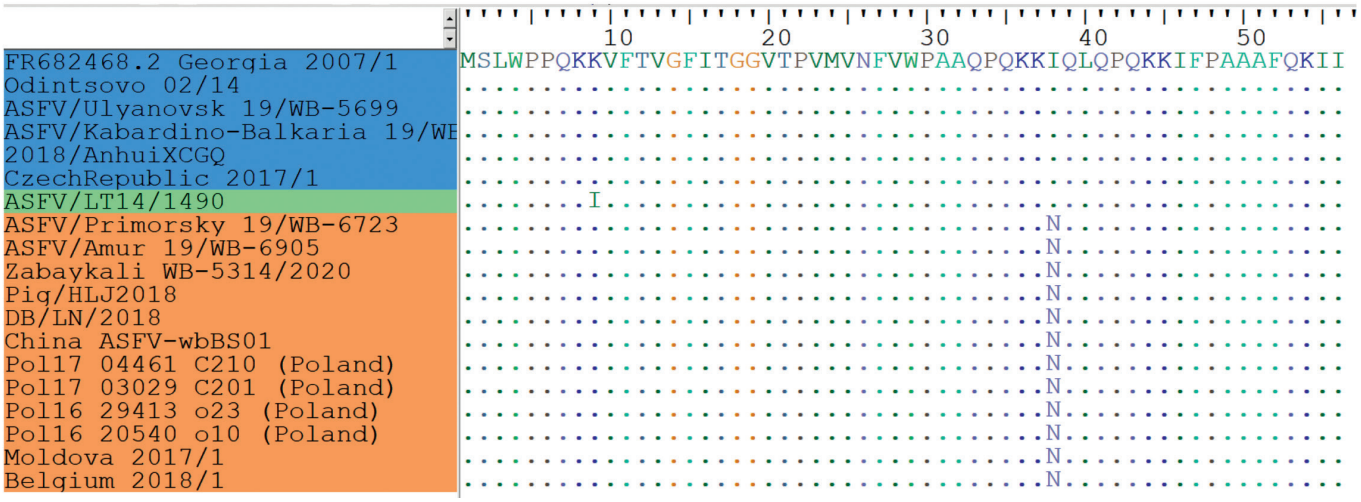

Fig. 4. Predicted amino acid sequences of the *DP60R* gene of African swine fever virus isolates.

Note. Each letter represents one amino acid according to international nomenclature. Isolates highlighted by different colors belong to different groups based on mutations in their sequences.

Рис. 4. Предсказанные аминокислотные последовательности продукта гена *DP60R* изолятов вируса африканской чумы свиней.

Примечание. Каждая буква представляет одну аминокислоту в соответствии с международной номенклатурой. Изоляты, выделенные разными цветами, относятся к разным группам в соответствии с мутациями в их последовательностях.

Fig. 5. Phylogenetic tree indicating the relationships between the combined amino acid sequences of the *MGF505-9R*, *C84L*, *I267L*, and *DP60R* genes of the isolates of African swine fever virus (*n* = 15).

Рис. 5. Филогенетическое древо, указывающее на связь объединённых аминокислотных последовательностей генов *MGF505-9R*, *C84L*, *I267L* и *DP60R* изолятов вируса африканской чумы свиней (*n* = 15).

Conclusion

Thus, a new variable region represented by the *DP60R* gene was identified using the ASFV complete genome sequencing. The clustering results based on this region were consistent with the results obtained by the analysis of full-length genomes. It can be assumed that further studies in clustering of ASFV isolates based on the analysis of the above gene sequence can put a spotlight on the significance of this gene for scientific research on evolution of the pathogen and its transmission paths.

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