Infection, Genetics and Evolution 71 (2019) 31–35

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Short communication

Detection and molecular characterization of novel porcine parvovirus 7 in Anhui province from Central-Eastern China

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ARTICLE INFO

Keywords:
Anhui province
PPV7
Phylogenetic analysis
Molecular epidemiology

ABSTRACT

Porcine parvovirus 7 (PPV7), a new serotype of the porcine parvovirus, was discovered in swine of the USA in 2016. Recently, PPV7 was detected in Anhui province, China. Twenty-four of the 120 lung samples were PPV7-positive. Three PPV7 strains were sequenced and named PPV7/China/AHbz, PPV7/China/AHhf, and PPV7/China/AHmas, respectively. The complete genome and NS1 gene nucleotides of the three PPV7 strains showed 80.0%–98.4% and 94.4%–98.7% sequence identity, respectively, to the other PPV7 strains obtained from NCBI. The three PPV7 strains from Anhui share a common origin with a PPV7 GX49 strain isolated in Guangxi. These results help to understand the molecular epidemiology of PPV7.

Parvoviruses are small, non-enveloped, single-strand linear DNA viruses capable of infecting many species (Schirtzinger et al., 2015). The family *Parvoviridae* is divided into two subfamilies based on the hosts – the *Parvoviridae*, that infect the vertebrates and the *Densovirinae*, that infect the arthropods. Currently, parvovirus contains eight genera: *Copiparvovirus, Tetraparvovirus, Erythroparvovirus, Dependoparvovirus, Aveparvovirus, Bocaparvovirus, Amdoparvovirus, and Protoparvovirus* (Palinski et al., 2016). The parvoviral genome is approximately 4–6 kilobases (kb) in length and typically composed of a non-structural protein (NSP) encoding a replicase (REP) activity and a capsid protein. The terminal sequences of the virus can form a palindromic hairpin structure (Streck et al., 2015).

Porcine parvovirus type 1 (PPV1) belongs to the genus *Protoparvovirus* and was first isolated from a cell culture contaminant in Germany in 1965 (Mayr et al., 1968; Mayr and Mahnel, 1964). PPV1 is considered to be one of the major causes of reproductive failure in pigs, causing huge economic losses in the global pig industry. In the past two decades, with the development of molecular biology techniques, six new serotypes of parvovirus have been clinically discovered in pigs and designated as Porcine parvovirus (PPV) 2 to PPV7(Cui et al., 2017; Palinski et al., 2016). PPV7 was first discovered in the USA in 2016 as a new serotype of porcine parvovirus in the rectal swabs of healthy adult pigs using metagenomic sequencing technology. The PPV7 genome is approximately 4100 bp in length and includes two open reading frames (ORF), a partial 5′ UTR and a 3′ UTR. The left ORF encodes the non-structural protein NS1 and the right ORF2 encodes the structural protein (Cap) (Palinski et al., 2016). There is currently very little information on PPV7, and cases of PPV7 infection has only been reported in USA, Sweden, Poland, and China (Blomstrom et al., 2018; Milek et al., 2018; Palinski et al., 2016; Xing et al., 2018). In China, PPV7 was first reported in Guangdong Province in 2017.

The PCV2 infection has been associated with a variety of clinical diseases and is known to cause immune suppression, causing porcine circovirus-associated disease (PCVD) (Li et al., 2016). PCV3 is a novel porcine circovirus that was first detected in the USA by metagenomic sequencing in 2016. It is associated with porcine dermatitis and nephropathy syndrome and reproductive failure (Palinski et al., 2017). We collected 120 lung samples from four commercial farms in Anhui Province. Diseased pigs have different clinical symptoms such as respiratory symptoms and gastrointestinal symptom. Each sample was tested for PCV2 and PCV3, using primers used in previous studies (Jiang et al., 2017; Ku et al., 2017). Viral nucleic acids were extracted from the lung tissue using the TIANamp Virus DNA Kit (TIANGEN BIOTECH, Beijing, China) following the manufacturer's instructions. The viral

Abbreviation: PPV7, porcine parvovirus 7; ORF, open reading frame; RDP4, Recombinant Detection Program version 4; PCV2, porcine circovirus 2; PCV3, porcine circovirus 3

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https://doi.org/10.1016/j.meegid.2019.03.004
Received 8 November 2018; Received in revised form 9 March 2019; Accepted 9 March 2019
Available online 12 March 2019

Table 1
Primers used for PPV7 detection and whole genome sequence amplification.

Primer	Sequence (5′ to 3′)	Expected length for product(bp)	PCR conditions	Reference	Purpose
			Denaturation Annealing Extension		
PPV7-F	CACGTTCATGGCTTACTGG	384	94 °C 30s 60 °C 30s 72 °C 25 s	This study	Detection
PPV7-R	AGGGACGAGACCTTCTTTGT				Genome sequencing
PPV7–1-F	GGAACGACAAGGACGACACTTCGAG	485	98 °C 10s 58 °C 45 s 72 °C 30s	This study	
PPV7–1-R	ATCGCCTCTTGTCTTGTCTGGTTAC				Genome sequencing
PPV7–2-F	GTGGTGGGGCTGGCTGAGCTG	977	98 °C 10s 58 °C 45 s 72 °C 60s	Xing et al., 2018	
PPV7–2-R	GCGTTGTCTTGCATGGACCAG				Genome sequencing
PPV7–3-F	ACACAAGCCGGGATTCCAGCA	1013	98 °C 10s 58 °C 45 s 72 °C 70s	Xing et al., 2018	
PPV7–3-R	CCACGAGCACTCCATCCCCTC				Genome sequencing
PPV7–4-F	ATCATCATGACGACCAACCACGCAC	831	98 °C 10s 58 °C 45 s 72 °C 50s	This study	
PPV7–4-R	AGGCGCTTTATTGATCACCGAAGC				Genome sequencing
PPV7–5-F	GGCGTCCGAAGAATCAGGGGTGGGT	686	98 °C 10s 60 °C 45 s 72 °C 45 s	This study	
PPV7–5-R	TTGGGCTCCGGTTCCTTTGCCGGTG				Genome sequencing
PPV7–6-F	ACAGCACCCGAGACGAACTGGACAT	950	98 °C 10s 56 °C 45 s 72 °C 60s	This study	
PPV7–6-R	TGGCGTTGAGAAGACACTGGTTTAG				

DNA obtained was used for detecting PPV7 by PCR using primer pairs shown in Table 1. The primers did not cross-react with the templates of PCV2, PCV3, PEDV, or PRRSV stored in our laboratory. A 20 μl PCR reaction mixtures contained 10 μl Premix Taq (Takara Bio Inc., Shiga, Japan), 1 μl of each 10 μM primer, 1 μl DNA template, and 7 μl ddH_2O. The PCR conditions were as follows pre-denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 25 s and a final extension at 72 °C for 10 min. The resulting PCR products were analyzed by 1% agarose gel electrophoresis.

The PPV7 genomic DNA was amplified using specific primers according the PPV7 GX48 strain (GenBank: MG543469.1). A total of six primer pairs were used, the sequence and the reaction conditions are presented in Table 1. The total reaction volume of 50 μl contained 25 μl Premix Taq (Takara Bio Inc., Shiga, Japan), 2.5 μl extracted DNA, 2.5 μl 10 μM forward primer, 2.5 μl 10 μM reverse primer, and 17.5 μl ddH_2O. After analysis by 1% agarose gel electrophoresis, the amplified PCR product was cloned into pMD19-T vector (TaKaRa, Dalian, China), transfected into DH5α competent cells, and then streaked onto LB agar plates containing 100 μg/ml ampicillin. The purified positive recombinant plasmid was sent to Sangon Biotech Shanghai Co, Ltd. for sequencing. The obtained sequences were assembled by DNAMAN software and other reference sequences downloaded from GenBank were aligned using DNASTAR software (DNASTAR Inc., Madison, Wisconsin, USA). A phylogenetic tree was constructed using the maximum-likelihood method with 1000 bootstrap replicates implemented in Molecular Evolutionary Genetics Analysis software MEGA 7.0.

Previous studies (Xing et al., 2018) have shown no significant recombination events between two distinct PPV genotypes, suggesting that recombination does not lead to a genetic diversity of PPV7. Frequent recombination between different parvoviruses has long been observed (Shackelton et al., 2007). Therefore, alignment of the genome was analyzed using Recombinant Detection Program (RDP) version 4 and the identified recombinant phylogenetic evidence was used to test whether PPV7 recombined between other known parvovirus. In addition, a similarity plots analysis was performed by the sliding window method of 200 bp and a step size of 20 bp as implemented in the SimPlot v. 3.5.1 package using a two-parameter (Kimura) distance model.

PPV7 were detected positive of the three of four pig farms. The positive rates of PCV2, PCV3 and PPV7 in these samples were 45.8% (55/120), 17.5% (21/120) and 20% (24/120), respectively. The co-infection rate of PCV2 and PPV7 was 17.5% (21/120), while the co-infection rate of PCV3 and PPV7 was 9.1% (11/120). The mixed infection of three pathogens (PCV2, PCV3, PPV7) reached 6.6% (8/120). Previous studies(Ellis, 2014).reported that PCV2 mixed infection with multiple pathogens is ubiquitous. The failure of PCV2 vaccination is believed to be an important reason for the emergence of PCV2 (Reiner et al., 2015; Xiao et al., 2012). In addition, PCV3 is also associated with reproductive failure. Identification of PPV7 mixed infection requires a more far-reaching investigation. Single infection with PPV7 was not detected in any sick-pig sample.

We detected three PPV7 strains in three different locations in Anhui. Then we amplified the PPV7 genome by PCR and obtained the whole genome sequence of three strains, named PPV7/China/AHbz, PPV7/China/AHmas, and PPV7/China/AHhf, respectively. Sequence are uploaded in GenBank under the accession numbers (MK484100, MK484101, MK484102). The nucleotide similarity between the three strains was 95.3%–98.4%. We obtained 19 known PPV7 genome sequences (Accession numbers: KU563733.1, MG543469.1, MG543460.1, MG543470.1, MG543464.1, MG543463.1, MG543472.1, MG543459.1, MG543457.1, MG543471.1, MG543469.1, MG543458.1, MG543456.1, MG543465.1, MG543466.1, MG543461.1, MG543468.1, MG543462.1, and MG543467.1) from NCBI, showed 80.0%–98.4% nucleotide identity with three isolates (data not shown). The phylogenetic tree based on the NS1 gene for the three Anhui isolates with 52 strains from different genera of the family *Parvoviridae*, revealed that the PPV7 Anhui isolate and the emerging PPV7 strain was in one branch and separated from other genera (Fig. 1.A). The phylogenetic tree based on the complete genome sequence showed that PPV7 is closely related to the Simian parvo-like virus 3 and Simian parvo-like virus 1, with Murine chapparvovirus, *Desmodus rotundus* parvovirus, Turkey parvovirus, and Bat parvovirus together in a branch (Fig. 1.B).

We used RDP 4 to analyze recombination between PPV7 isolates, Simian parvo-like virus 3, Simian parvo-like virus 1, *Desmodus rotundus* parvovirus, Murine chapparvovirus, Turkey parvovirus, Bat parvovirus, and other parvoviruses. The results of the analysis did not reveal any recombination signals for PPV7. Moreover, no recombination events were identified using analysis using the Simplot package (Fig. 2). The results of both analyses are consistent, indicating that PPV7 may not be produced through recombination of the currently known parvovirus.

Overall, our results indicate that PPV7 is widely distributed in Anhui Province in Central-eastern China. We did not detect single pathogen infection with PPV7 in any of the samples, suggesting that PPV7 may be a latent infection. Recombinant analysis revealed that PPV7 was not recombined with any known parvoviruses. Further research is warranted to elucidate the pathogenesis of this new serotype of porcine parvovirus.

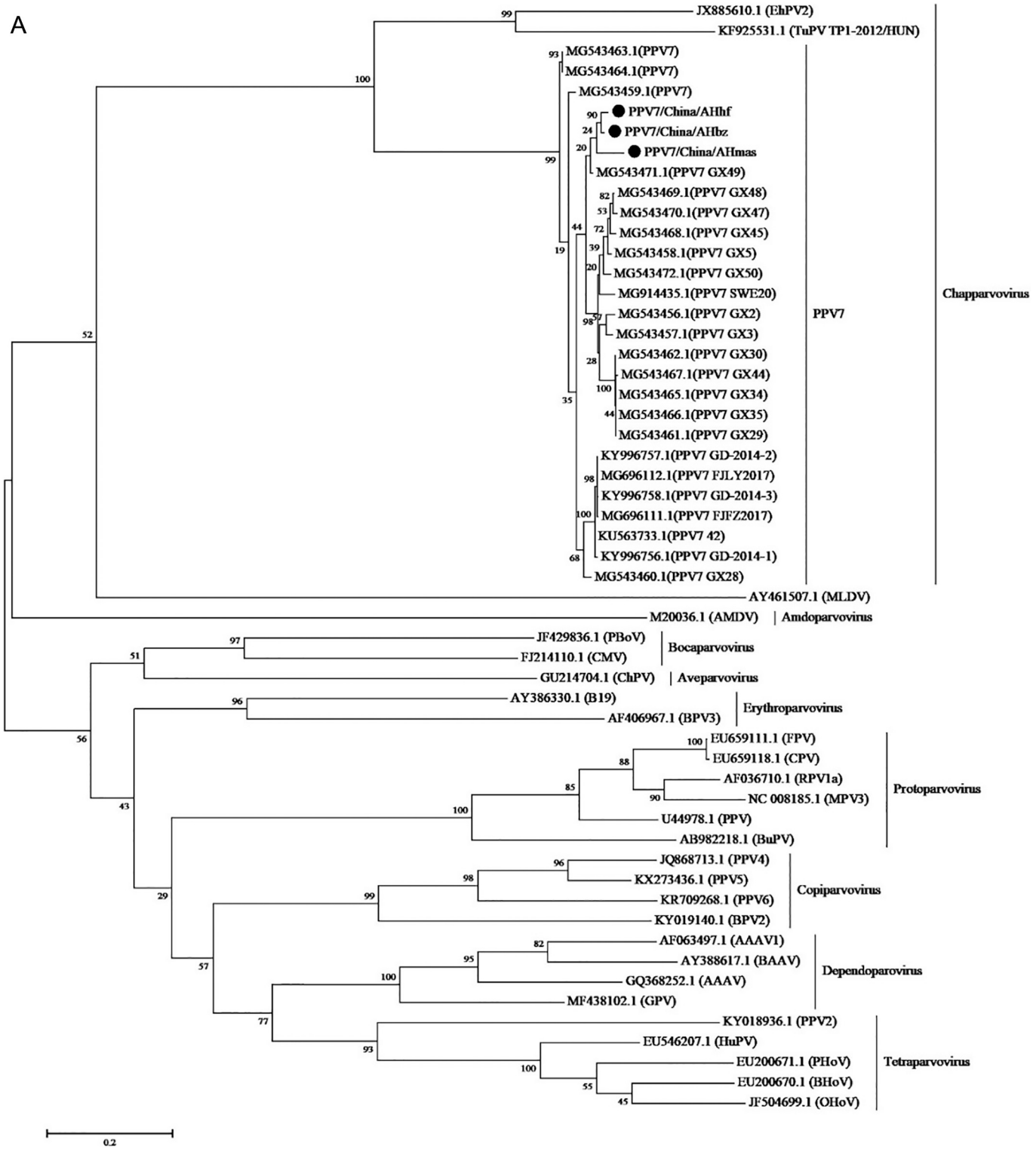

Fig. 1. (A) Phylogenetic tree constructed based on the NS1 gene of PPV7 strain ($n = 52$) and 3 strains of PPV7 Anhui isolate. (B) Phylogenetic tree based on the whole genome of different parvovirus species. The tree reliability was assessed using the maximum-likelihood method with the Tamura-Nei model and 1000 bootstrap replications. The strains detected in our laboratory are marked with a black circle.

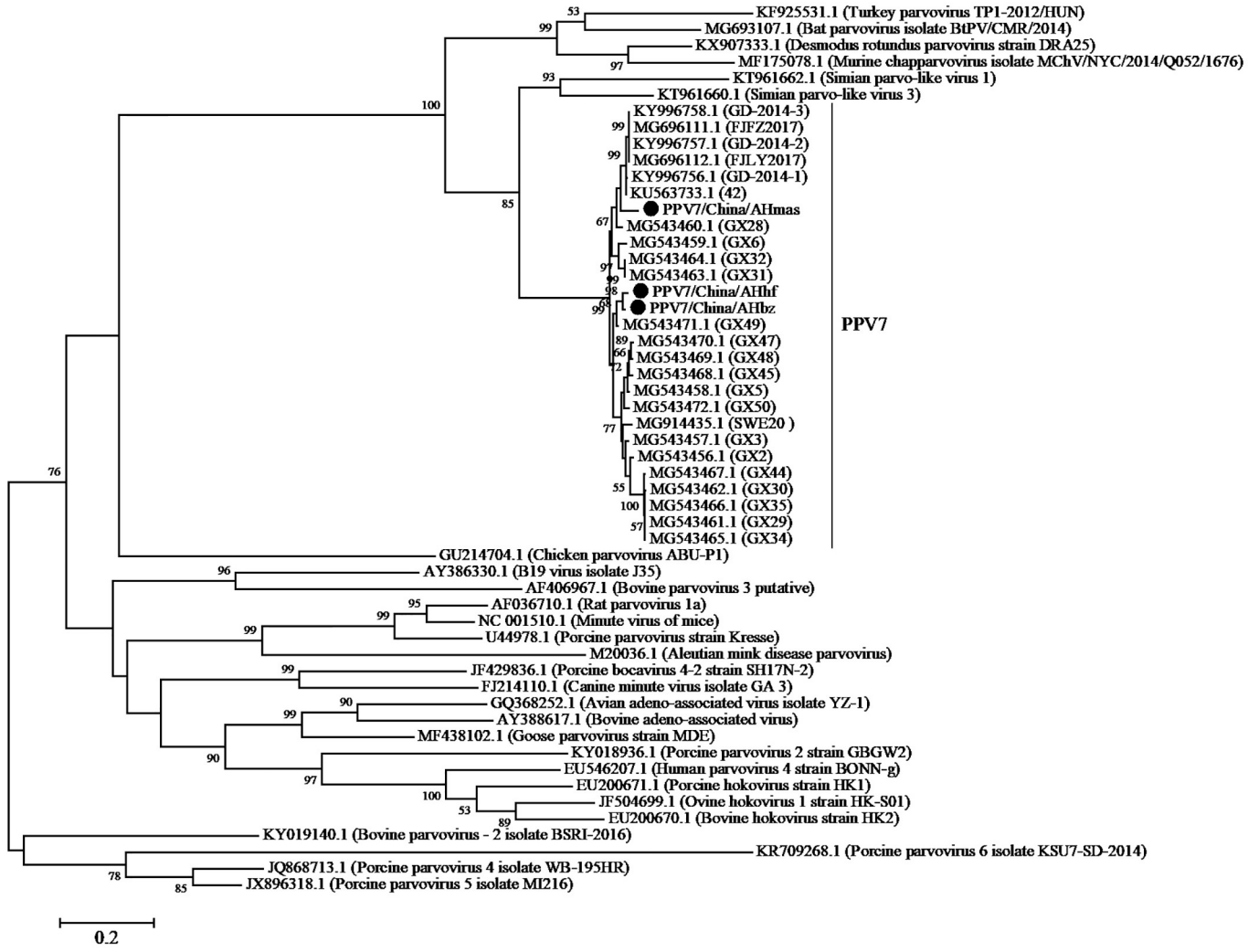

Fig. 1. (*continued*)

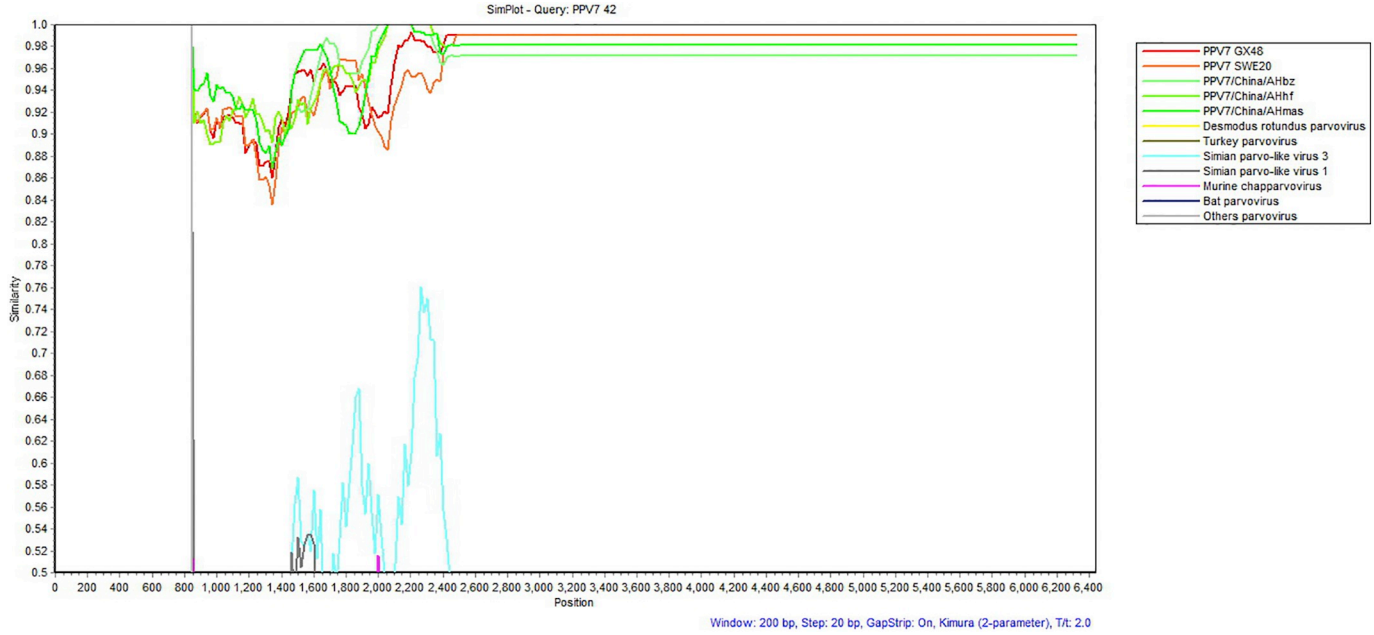

Fig. 2. Similarity plot of whole genomes of 32 different parvovirus species. The isolate from the USA, PPV7 42 strain (Accession number: KU563733.1), was set as the query strain. The vertical and horizontal axes represent the nucleotide similarity percent and nucleotide position (bp) of the alignment in the graph, respectively.

Declaration of conflicting interests

The authors declare that they have no conflict of interest.

Acknowledgments

This work was supported financially by the National Natural Science Foundation of China (No. 31602063), Anhui Provincial Natural Science Foundation (No.1508085QC60).

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