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Deciphering the Virome of the Pimple-Shaped 'Yali' Pear Fruit through High-Throughput Sequencing

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Abstract: Viral diseases pose a threat to fruit tree growth. In this study, we observed some pimple-shaped 'Yali' pears (*Pyrus bretschneideri* Rehd.) and investigated their viral components. We used virome analysis to elucidate the viral composition within the pimple-shaped fruit. RT-PCR was applied to detect the plant viruses of fruits, leaves, and branches in 'Yali' pear. We also constructed a phylogenetic tree based on the amino acid sequences of the movement proteins of 6 apple stem grooving virus (ASGV) isolates and 44 ASGVs from the NCBI database. We detected ASGV and apple stem pitting virus (ASPV) in the pimple-shaped pear fruits, which is the first report of these viruses existing in 'Yali' pear fruits. ASGV was present in all pimple-shaped fruit samples from six 'Yali' pear-producing regions. The phylogenetic tree showed that ASGVs from pears, apples, and citrus plants were separated into different branches, suggesting that hosts influence the genetic diversity of ASGV. Our study revealed the viral components and genetic variation of ASGV in pimple-shaped pear fruit, providing new insights into the epidemiology of this virus.

Keywords: pear fruit; virome; apple stem grooving virus (ASGV); genetic variation

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1. Introduction

Viral diseases are a major threat to the growth, yield, and quality of fruit trees, as well as the safety of the fruit industry [1]. Depending on their symptoms, viruses can be classified into non-cryptic and cryptic types. Cryptic viruses are often asymptomatic at the initial stage of infection and do not attract much attention. However, they can spread widely through pruning and grafting operations, causing large-scale infections that impair the growth and development of fruit trees [2].

More and more viruses are being discovered in fruit trees due to the development of detection technology. In citrus crops, more than 20 viruses have been identified and reported, including citrus tristeza virus (CTV) [3]; citrus psorosis ophiovirus (CPV) [4]; citrus leprosis virus C, C2, and n (CiLV-C, -C2, and -N) [5,6]; citrus vein execution virus (CVEV) [7]; citrus leaf blotch virus (CLBV) [8]; citrus yellow vein cleaning virus (CYVCV) [9]; citrus yellow mosaic virus (CYMV) [10], etc. In pome fruits, encompassing apples and pears, several viruses occupy a prominent position. These include apple stem grooving virus (ASGV) [11], apple stem pitting virus (ASPV) [12], and apple chlorotic leaf spot virus (ACLSV) [13], all members of the *Betaflexiviridae* family. These viruses exhibit comparable molecular and biological characteristics. Typically, they remain latent in the majority of commercially cultivated apple varieties, yet they have the potential to manifest as disease when grafted onto sensitive rootstocks. Distinct from these, apple mosaic virus (ApMV) possesses a broader host range, infecting numerous woody plants [14]. It is often found co-existing with ACLSV, ASPV, ASGV, and other apple-infecting viruses in mixed infections. The affected leaves may be scattered randomly throughout the tree or confined to a single

branch, often leading to premature leaf fall. Notably, ApMV remains asymptomatic in pear [15].

In China, the most common viruses and viroids in pear leaves and branches are ASGV, ASPV, apple dimple fruit viroid (ADFVd), and ACLSV. Recent research has revealed that fruit trees may harbor the ASGV virus [16]. Wang et al. [17] used indicator plants, including *Pyrus communis*, Berre Hardy, Doyenne du Comice, *Pyronia veitchii*, and *Cydonia oblonga*, to identify viruses on the leaves of six different species of pears in the field from Northeast China and found that most of the pear trees examined had ASGV. Tan et al. [18] detected ASGV on apple leaves and discovered that it was prevalent in the main apple-planting regions of Northwest China. Youssef et al. [19] used RT-PCR and ELISA to pinpoint the widespread presence of ASGV and ASPV in the petiolar region of pear trees in Egypt. Yin et al. [20] tested virus species in test tube plantlets of three major cultivars of early pear in South China and identified ASGV on regenerated seedlings. Kim et al. [21] collected leaf samples from 35 Korean pear orchards and performed RNA sequencing and phylogenetic analysis, revealing that ASGV and ASPV were the predominant viruses. However, none of these studies reported the virus carriage of pear fruit.

The study of plant viriome can provide a more comprehensive and in-depth understanding of the species and diversity of viruses in plants [22]. More and more viruses are being discovered through viromic techniques. Recent research has expanded our understanding of the plant virome. Yang et al. [23] investigated the virome of 161 plant species from 38 orders in a riverside ecosystem, revealing a complex viral community and expanding our understanding of plant-associated viral diversity and host ranges. The virome of tomato has applications beyond discovering and detecting viruses in tomato within a specific geographic area; it can also reveal the virome of nearby plants and vectors [24]. Tabara et al. [25] found a high prevalence of asymptomatic tobacco ringspot virus (TRSV) infection in melon fruits and a large accumulation of viral transcripts by using phylogenetic analysis, RT-PCR, and RNA sequencing.

'Yali' pear (*Pyrus bretschneideri* Rehd.) is one of the most widely cultivated and consumed pear varieties in China and is favored by consumers for its attractive fruit shape, crisp and juicy flesh, and storage performance [26]. In recent years, we observed some pimple-shaped fruits in various 'Yali' pear planting regions. Therefore, there is an urgent need to investigate the viral components and diversity in pimple-shaped pear fruits. Here, we applied viromic technology and RT-PCR methods to explore the viral components and diversity in these fruits, and also analyzed the genetic variation of ASGV isolates based on the amino acid sequences of movement proteins, to provide new insights for the epidemiology of this virus.

2. Materials and Methods

2.1. Sample Collection

The 'Yali' pear leaves and branches were collected from the orchard in Wei County, Hebei Province, and the pimple-shaped 'Yali' pear fruits were collected from orchards in Wei County (W), Shenzhou City (S), Jinzhou City (J), Zhao County (Z), Botou City (B), and Xinji City (X), which are the main pear-producing regions in Hebei Province. The sampling method for each site was as follows: three symptomatic trees were randomly selected from a diseased orchard, and ten leaves, ten branches, and ten fruits were taken from each tree, each forming a mixed sample of leaves, branches, and fruits. After collection, the samples were immediately packed in separated fresh-keeping bags and transported to a laboratory.

2.2. Extraction of Total RNA

One mixed sample consisting of ten symptomatic samples was used for the experiment. Intact leaves, branches attached to fruits, and the pimple-shaped parts of fruits were ground and used for RNA extraction. Briefly, the tissues pre-cooled with liquid nitrogen were ground to a uniform powder using a mortar and pestle, and then 100 mg of the powder was weighed into RNase-free centrifuge tubes for total RNA extraction using

the RNAprep Pure Polysaccharide Polyphenol Plant Total RNA Extraction Kit (Tiangen, Beijing, China), and the procedure was carried out strictly according to the instructions. Three samples were used to extract RNA in parallel. RNA integrity was evaluated using agarose gel electrophoresis.

2.3. Virome Analysis

The total RNA of pimple-shaped pear fruits was extracted as above. Genomic DNA was removed from the samples using FastKing RT Kit (With gDNase) (KR116, Tiangen, Beijing, China) and cDNA was synthesized according to the instructions. cDNA was sequenced using Illumina NovaSeq platform at Personal Biotechnology, Shanghai, China. The cDNA synthesized from the enriched mRNA was randomly fragmented into short pieces, and the insert libraries of suitable length were sequenced by paired-end (PE) sequencing. Adapters containing unique barcodes were ligated to the ends of the cDNA fragments, which were necessary for PCR amplification and for the subsequent attachment to the sequencing platform's flow cell. The samples were loaded onto the Illumina platform once the libraries had been prepared.

The database was constructed using viral genome data from NCBI with the Kraken software (Kraken 2) [27], and the confidence threshold was set to 0.5 for annotating the sequences obtained. The circos diagram was performed by the genescloud tools, a free online platform for data analysis (https://www.genescloud.cn, accessed on 15 March 2024). The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive [28] in the National Genomics Data Center [29], China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA010784) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa (accessed on 25 April 2023).

2.4. Identify Virus Species by RT-PCR

cDNA was then used as a template for further amplification of specific sequences using specific primers (Table 1). The amplification conditions were as follows: denaturation at 98 °C for 3 min, 20 s at 98 °C, 20 s at annealing temperature (ASGV 50 °C, ASPV 50 °C, ADFVd 53 °C, ACLSV 43 °C), 20 s at 72 °C for 35 cycles, extension at 72 °C for 5 min, and storage at 10 °C. The PCR products were separated by 1% agarose electrophoresis, and the products with correct size were recovered by a DNA gel recovery kit (Axygen, Union City, CA, USA), followed by Sanger sequencing at Sangon Biotech (Shanghai, China) Co., Ltd. (Shanghai, China). The virus species was determined by an online comparison of sequencing results at NCBI (National Center for Biotechnology Information).

Table 1. Information of primers.

Names	Sequences	Size of Fragments	Sources
ASGV-F ASGV-R	AAGAGAGGATTTAGGTCCCTC ATAAAGGGAGGCATGTCAACC	825 bp	[30]
ASPV-F ASPV-R	TGCCTCAAAGTACACCCCTCAGT CGCCAAGAAATGCACAGC	316 bp	[30]
ADFVd-F ADFVd-R	GAGGAAAACTCCGTGTGGTTC AAGTCCACTCCCTGCCAGACC	271 bp	[30]
ACLSV-F ACLSV-R	CAGACCCTTATTGAAGTCGAA GGCAACCCTGGAACAGA	358 bp	[30]
ASGV-MP-F ASGV-MP-R	ATGGCTATCGTCAACGTCAACCG TCAGGGGGAGGAACCGTCAGAAG	963 bp	This Study

2.5. Amplification and Sequencing of the Full Sequence of Movement Protein Gene

Primers were designed based on the reported full sequence of the coding gene sequence of movement protein in ASGV, and the PCR conditions were set as follows: initial denaturation at 98 °C for 3 min; 35 cycles of denaturation at 98 °C for 20 s, annealing at 60 °C for 20 s, and extension at 72 °C for 20 s; final extension at 72 °C for 5 min; and storage at 10 °C. The band size was confirmed by agarose gel electrophoresis, and the T vector was ligated using the pEASY®-T1 Cloning Kit (TransGen, Beijing, China). The positive clones were sent to Shanghai Biotech Biological Co. (Shanghai, China) for sequencing.

2.6. Analysis of Molecular Variation of ASGV

The ASGV movement gene and protein sequences were retrieved from the database and analyzed using CLC Sequence Viewer version 8.0 (QIAGEN, Hilden, Germany) for protein sequence alignment. The Recombination Detection Program software (RDP4) was used to detect the homologous recombination. The *p*-value of the highest multiple comparison accuracy (multiple-comparison-corrected *p*-value) was set to 0.01. Then, a phylogenetic tree was constructed by CLC Sequence Viewer version 8.0 (QIAGEN, Germany). The default parameters were used for protein sequence alignment, and the neighbor joining method was used for phylogenetic tree construction with 1000 bootstrap replicates.

3. Results

3.1. Virome Analysis of Pimple-Shaped Fruits in 'Yali' Pear

We have noticed that many fruits with pimple-shaped are frequently found in 'Yali' pears in recent years. The main symptoms of these abnormal pears showed many abnormal bumps, depressions or grooves on the fruit surface, resulting in a pimple-shaped appearance. As shown in Figure 1, some pears in the orchard were observed to be pimple-shaped on the tree, which seriously affects the appearance and quality of the fruits.

Figure 1. Symptoms of pimple-shaped pear fruit in 'Yali' pear.

To elucidate the viral composition within the pimple-shaped fruit, we sequenced the total RNA within the pimple-shaped 'Yali' pear fruit using virome analysis. Table 2 shows the sequencing quality of the three samples, including the sequencing volume, the fraction of high-quality bases, and other data. We compiled the sequencing offline raw data of each sample to obtain these results. The results showed that the minimum number of reads in the samples was 76,158,416, covering 11,423,762,400 bases. Therefore, the sequencing depth of this study was quite large, enough to meet the analysis of virus composition in pimple-shaped pear fruits.

To identify the virus species present in the pimple-shaped pear fruit, we aligned and annotated the obtained sequences in the NCBI virus database, as shown in Figure 2. We identified four virus species, ASPV, *Capillovirus* sp., ASGV, and *Foveavirus* sp., with respective proportions of 38.3%, 31.3%, 22.6%, and 7.8% among the three samples. *Capillovirus* sp. and *Foveavirus* sp. are virus species within the genera *Capillovirus* and *Foveavirus*, respectively, which have not been classified into specific species. Because ASGV belongs to the *Capillovirus* genus, whereas ASPV belongs to the *Foveavirus* genus, the relative viral

abundance of *Capillovirus* (53.9%) is higher than that of *Foveavirus* (46.1%) at the genus level. It is worth noting that pear fruits were not previously reported as organs infected by ASGV and ASPV, and this study aimed to investigate virus infection in pimple-shaped 'Yali' pear fruits in response to this new discovery.

Table 2. Statistics of RNA-sequencing data in pimple-shaped pear fruit.

Sample ID	Read Number	Total Number of Bases Measured (bp)	N (%)	GC (%)	Q20 (%)	Q30 (%)
wx1	76,158,416	11,423,762,400	0.00061	44.9	97.86	94.68
wx2	76,758,390	11,513,758,500	0.00062	44.79	97.51	94.19
wx3	81,919,920	12,287,988,000	0.00061	44.81	97.72	94.28

Note: N (%) is the percentage of fuzzy base in total base; GC (%) is the percentage of GC content, that is, the percentage of the sum of G and C bases in the total number of bases; Q20 (%) and Q30 (%) are the percentage of the bases with an accuracy of more than 99% and 99.9% in the total bases, respectively.

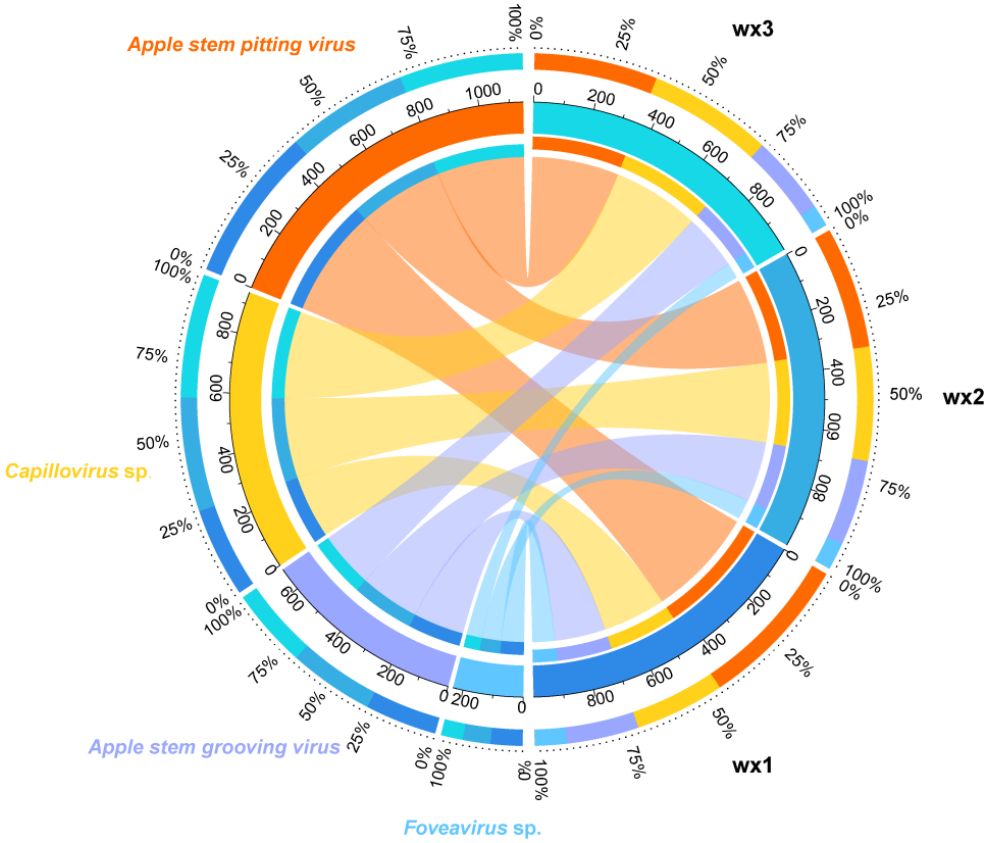

Figure 2. Virus composition of pimple-shaped fruit in 'Yali' pear. Circos diagram is used to show the relationship between viruses and samples, showing 5 circles of information from the outside in turn. Circle 1: species and sample information. The names of species are shown in different colors. Circle 2: the percentage of Amplicon Sequence Variants (ASV) relative abundance in the sample or species. Circle 3: ASV and the main block of the sample are distinguished by different colors. The scale outside the block is the absolute abundance information of ASV. Circle 4: ASV and sample sub-block, corresponding to the main block, showing the abundance of each ASV in each sample and the abundance information of each ASV contained in each sample. Circle 5: Line showing ASV and sample association information, corresponding to ASV and sample sub-block.

3.2. Analysis of Virus Infection in Different Organs of 'Yali' Pear

To further verify the distribution of plant viruses, we used RT-PCR to detect four plant viruses including ASGV, ASPV, ADFVd, and ACLSV in pimple-shaped fruits, leaves, and branches. The results showed that ASGV was detected in all samples, suggesting that ASGV was widespread (Figure 3). ASPV was also detected in nearly all the samples, although the intensity of PCR products on gel electrophoresis was not as high as ASGV. This indicates a higher prevalence of ASGV than ASPV in the pimple-shaped fruits, as well as in the leaves and branches of the 'Yali' pear in Hebei Province, aligning with the findings from the virome analysis.

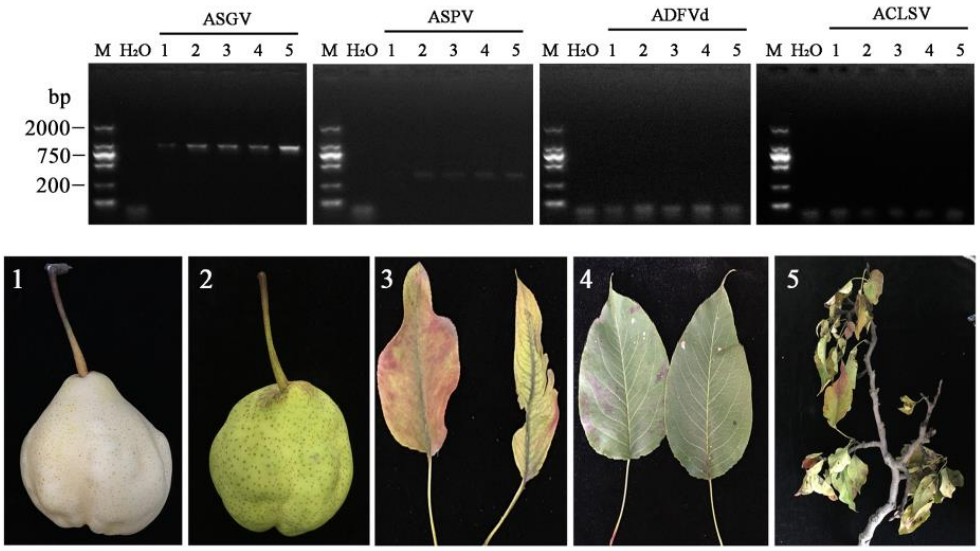

Figure 3. Detection of virus in different organs of 'Yali' pear. ACLSV represents apple chlorotic leaf spot virus, ASGV represents apple stem grooving virus, ASPV represents apple stem pitting virus, ADFVd represents apple dimple fruit viroid. M, marker; 1–2, pear fruit with pimple-shaped appearance; 3, diseased pear leaf; 4, normal pear leaf; 5, pear branch.

3.3. Detection of Virus Infection in 'Yali' Pear Pimple-Shaped Fruit

Based on the analysis of different organs of the pear tree, the results indicated that the pimple-shaped fruits also carried viruses. Since there are few studies on viral infection in the fruit, we conducted the detection of the virus in the pimple-shaped 'Yali' pear fruit from six different origins, named sample W, S, Z, J, B, and X. The results showed that ASGV was carried by pimple-shaped pear fruits from all sources, indicating that this virus had the most widespread range in pear fruits (Figure 4). Obvious ASPV-specific bands were detected only in S, indicating that this virus also existed in pear fruits, but its spread range was much smaller than that of ASGV. ACLSV and ADFVd were hardly detected in the pimple-shaped pear fruits from the investigated sources, indicating that they had not formed a large range of spread in pear fruits. These results were basically consistent with the virome analysis results; that is, ASGV and ASPV were the most common two viruses in the pimple-shaped pear fruits, and ASGV had the highest prevalence.

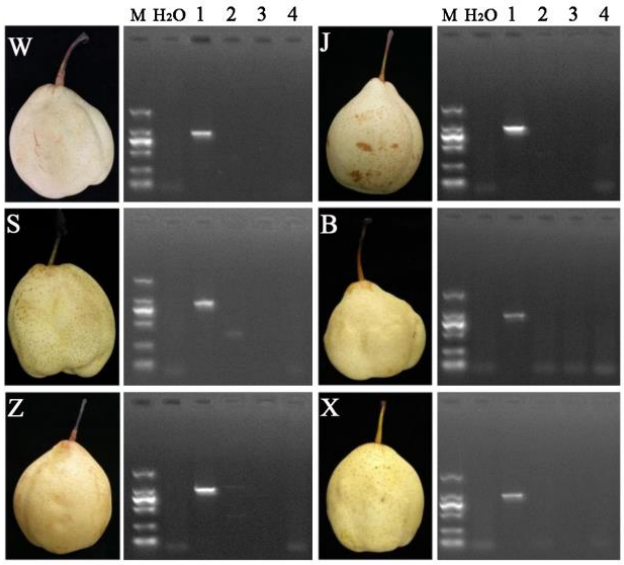

Figure 4. Detection of virus in pimple-shaped 'Yali' pear fruit from different regions, including Wei County (W), Shenzhou City (S), Jinzhou City (J), Zhao County (Z), Botou City (B), Xinji City (X) and M (marker); 1–4 represent ASGV, ASPV, ADFVd, and ACLSV.

3.4. Molecular Variation Analysis of ASGV Virus

We further determined the molecular variation of ASGV from different sources. Based on the reported genome of ASGV, primers that could amplify the full length of the MP gene were designed, and the PCR results showed that the MP gene of ASGV in fruits from six different regions could be amplified accurately, and the size of the target bands was consistent with the reported size of about 963 bp (Figure 5).

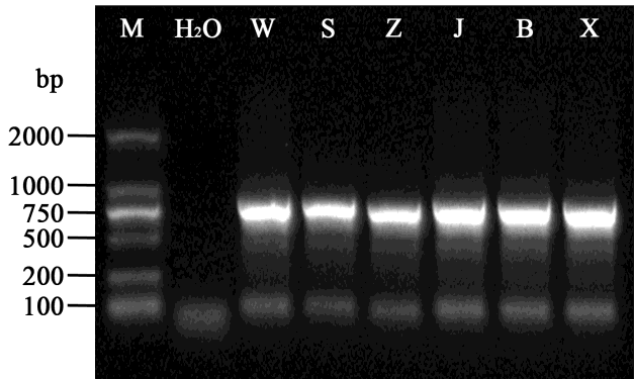

Figure 5. Full-length gene application of movement protein of ASGV in pimple-shaped 'Yali' pear fruit. M (marker), W (Wei County), S (Shenzhou City), J (Jinzhou City), Z (Zhao County), B (Botou City), X (Xinji City).

The amino acid sequences of the six ASGV strains identified in this study were further compared and analyzed with the reported movement proteins of two ASGV strains (Chinese apple AFG28409.1 and Indian apple CBY84302.1). As shown in Figure 6, the ASGV movement proteins among the six 'Yali' pear origins had high similarity and were also highly similar to the ASGV movement protein of Chinese apple (AFG28409.1), but differed from the ASGV movement protein in Indian apple (CBY84302.1). Geographical differences thus may be one strong factor influencing the molecular variation of ASGV.

Furthermore, in order to explore the evolutionary relationship of ASGV from pimple-shaped pear fruits with those from other regions and hosts, we searched for the sequences of ASGV genome in NCBI database and found 44 movement protein sequences of ASGV. The information of their sequences, hosts, and source regions are shown in Table S1.

The comprehensive RPD analysis of homologous recombination, conducted specifically on the MP genes of all viruses, conclusively demonstrated the absence of any homologous recombination events within the MP gene region. Then, a molecular phylogenetic tree was constructed using the amino acid and nucleotide sequences of movement proteins for ASGV, respectively. In total, 44 ASGVs from the NCBI database and 6 ASGVs from the different regions of pear fruits obtained in this study were applied (Figures 7 and S1). The results showed that the ASGVs from apple, pear, and citrus plants as hosts could be clustered into one class on some branches, indicating that the host specificity of ASGV had some influence on its molecular variation. The six ASGVs identified in this study were clustered under the same branch, indicating that the molecular variation of ASGV in pimple-shaped fruits in Hebei Province was small. However, the molecular variation of ASGV in pear fruits needs to be further detected and verified in the future with more samples from a wider range of sources.

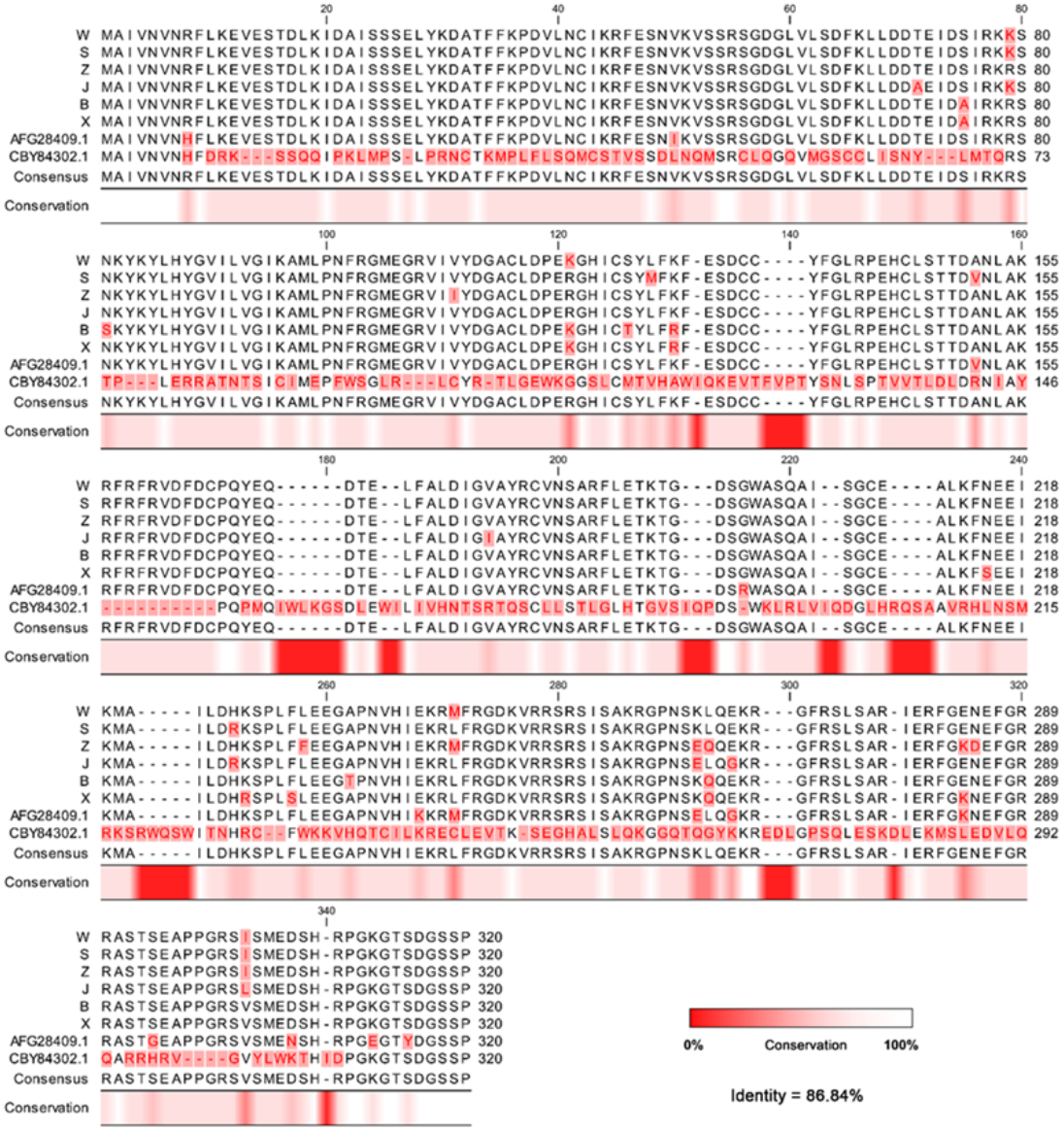

Figure 6. Alignment of amino acid sequence of movement protein of ASGV in pimple-shaped 'Yali' pear fruit. Differential amino acids are marked in red.

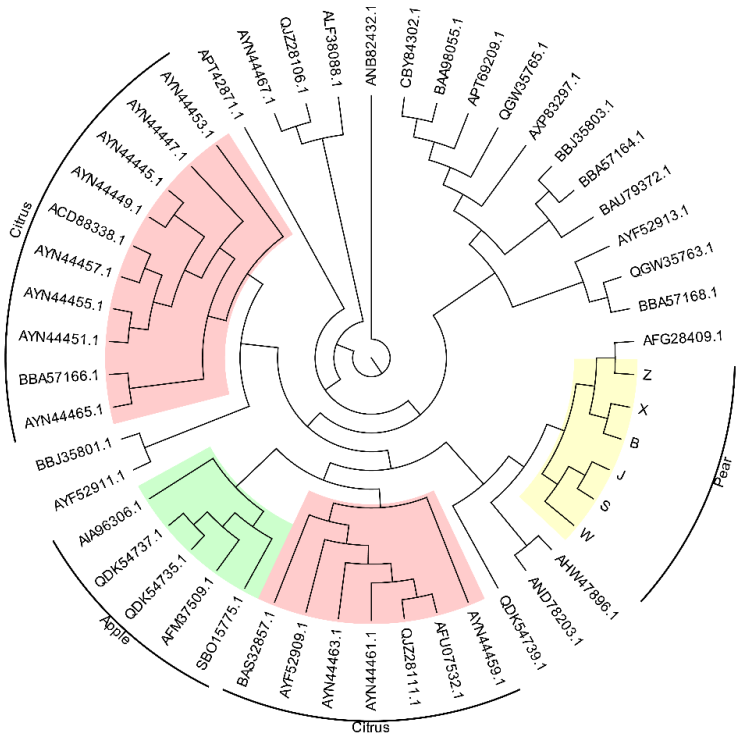

Figure 7. Evolutionary analysis of ASGV using protein sequence of movement proteins. Neighbor joining method was used for phylogenetic tree construction with 1000 bootstrap replicates. Citrus, apples, and pears are labeled in red, green, and yellow, respectively.

4. Discussion

Fruit tree virus transmission is common and poses significant production challenges as it is hard to prevent, treat, and eradicate [31]. Cryptic viruses, especially, can cause epidemics as they often do not show symptoms. Yao et al. [2] performed virus detection for 387 apple leaf samples from the provinces of Yunnan, Guizhou, and Sichuan and found that the positive rate of ASGV was 100%, ASPV was 89.4%, and ACLSV was 81.6%, indicating that most of the samples had mixed infections. Niu et al. [32] used the bark and young leaves of Korla pear (*Pyrus brestschneideri*) trees as materials and established the RT-PCR detection system for pear vein yellow virus (PVYV). Pear tree viruses have been detected in many organs of fruit trees. Using pear leaves, Li et al. [30] identified the cryptic virus in 70 pear trees of different varieties in three regions of Tianjin. ACLSV was found to be the main cryptic virus species. Sun et al. [33] used blossoms as test material to identify and analyze the complete sequence of ASGV in Korla pear. Gao et al. [34] detected a high rate of ASGV (100%) and many other viruses in pear rootstocks preserved in the resource nursery of Qingdao Agricultural University. However, research on pear fruits carrying plant viruses is still unclear. In this study, we found that pears in Hebei Province, China, frequently harbored plant viruses, and we reported the presence of ASGV and ASPV in the pimple-shaped fruits of 'Yali' pear for the first time. Moreover, there was often a co-infection of multiple viruses, suggesting that grafting and pruning had facilitated the wide spread of the cryptic virus.

Virome technology has been widely used in the identification, diagnosis, and evolutionary study of plant viruses since the development of sequencing technology [35]. In this study, we used viromics and RT-PCR technology to detect viruses in pimple-shaped fruits of the 'Yali' pear. It was found that the pimple-shaped fruits carried various plant viruses and often had co-infections, confirming that the pear fruits indeed carried multiple plant viruses. Yang et al. [36] used high-throughput sequencing technology to analyze the transcriptomes of the plant virus species in the blossoms of Korla pear. They found three viruses that have been reported in pears (ASGV, ASPV, and ACLSV), as well as brassica

yellowing virus (BrYV), which was reported for the first time in pears. By using high-throughput sequencing techniques, luteovirus infections were detected in both apple [37] and peach [38]. Therefore, viromic technology has broad potential for development and application in the field of plant virus research, especially in the study of fruit viruses and the discovery of new viruses. We performed viral composition analysis by virome analysis and identified two viruses commonly found in pears, ASGV and ASPV. This indicates that there are already a reasonable number of these viruses present in the pimple-shaped fruits of 'Yali' pear. Considering that pimple-shaped 'Yali' pears have been found in some pear-producing areas, further experiments are needed to investigate whether these viruses are related to the formation of fruit deformities and how they cause the occurrence of deformities.

Plant viruses colonized in pear fruits and the associated deformities are seldom reported, unlike those in leaves and rootstocks. The most notorious pear fruit disease caused by a virus is stony pit disease, and it impairs pear fruit development and leads to fruit depression and deformation [39]. This disease is predominantly reported in European pears, with varying symptom severity among different varieties, and is most common in regions where Bosc pears are cultivated [40]. At present, there are no reports of this disease in Chinese pears. In this study, we detected a high abundance of ASGV and ASPV in the fruits of the 'Yali' pear, a representative Chinese pear, which might be implicated in fruit deformation. Some reports have tried to associate ASPV and/or ACLSV as the pathogen of pear stony pit disease; however, none of them has reproduced the symptom of stony pit in pear fruit [41]. This is also where research on this disease urgently needs to be promoted.

The molecular variation of viruses is an important way that they maintain their high infectivity, especially for plant viruses with RNA as the main genetic material. It was found that the coat protein molecular variation of ASGV in Kuril balsam pear was not related to the host or the geographical region, while the movement protein molecular variation of ASGV was related to the host [33]. The molecular variation of ASGV in apple trees in Yunnan Province, China, had some association with the host/geographical origin [42]. The molecular variation of ASGV in 'Red Berryessa' (*Pyrus communis*) had no geographical correlation but had some host specificity [43]. Eight ASGV virus strains from different varieties of 'Xinjiang' pear (*Pyrus sinkiangensis*) and sand pear (*Pyrus pyrifolia* Nakai) were located in two different branches from three ASGV strains in red pear, and it was speculated that complex genetic variation might exist [44]. Evolutionary analysis based on the amino acid sequences of movement proteins from the 6 fruit ASGVs and 44 reported ASGVs showed that a significant number of ASGVs in apple, pear, and citrus plants had evolved into different branches, indicating that differences in viral hosts were one of the most significant factors in the genetic variation of ASGV. This is consistent with the findings so far reported based on genomic or coat proteins [45].

In summary, this study found that ASGV and ASPV were the main virus species in pimple-shaped fruits, and ASGV had the highest prevalence. ASGV and ASPV were not only carried by pear leaves and branches but by pimple-shaped fruits in 'Yali' pear. They had already been widely spread and detected in multiple regions. Six fruit ASGVs were identified, which were highly homologous with ASGVs from different host varieties and regions. Variety difference was the main factor affecting ASGV molecular variation.

5. Conclusions

ASGV and ASPV were prevalent in pimple-shaped fruits of 'Yali' pear from multiple regions in Hebei Province, and ASGV had the highest abundance according to the virome analysis. The six ASGV strains detected in 'Yali' pear fruits showed high homology with ASGV from various hosts and regions, but they also had some differences among them. Host diversity was the major factor influencing the molecular variation of ASGV. Future research should focus on using molecular and biochemical methods to reveal the relationship between these viruses and the formation of pimple-shaped pear fruits, including virus content, virus species, and the physiological state of the host. As the transmission of ASGV and ASPV is mainly facilitated by the planting of infected nursery stocks and through

traditional grafting methods, this study will provide more comprehensive information for the epidemiology of these diseases.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10040311/s1, Table S1: Movement proteins and properties of ASGV. Figure S1. Evolutionary analysis of ASGV using nucleotide sequence of movement proteins. Neighbor joining method was used for phylogenetic tree construction with 1000 bootstrap replicates.

Author Contributions: Conceptualization, J.G. and Y.Z.; methodology, Y.Z.; formal analysis, C.G. and Y.G.; data curation, Y.C. and C.W.; writing—original draft preparation, Y.Z.; writing—review and editing, J.G. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The raw data have been deposited in the Genome Sequence Archive in the China National Center for Bioinformation (https://www.cncb.ac.cn/, accessed on 25 April 2023) under the project number PRJCA016541.

Conflicts of Interest: The authors declare no conflicts of interest.

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