Metagenomic Analysis of Non-*Apis* Wild Insect Pollinators and Predators Shows Virus Threat to Their Survival

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Research

Keywords: Wild insect pollinators, Novel viruses, Viral threat, Pathogenicity

Posted Date: July 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-665378/v1>

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Abstract

Background: Insect pollinators provide major pollination services for wild plants and crops, this is necessary for both agriculture and ecology, we should protect their population size and diversity as much as possible. Currently, we have known that the virus disease of honeybee can cause serious damage to bee colony, but we know little about the virus of other wild pollinating insects. Here we investigated the virus host wild insect pollinators as a reminder that they are facing virus threaten too.

Methods: Transcriptome sequencing was used to investigate the viruses of Non-*Apis* insect pollinators and predators. Furthermore, host and replicability of several novel viruses that weakly related to honey bee viruses were determined by using reverse transcription-polymerase chain reaction (RT-PCR).

Results: Three honey pathogenic virus, five viruses host insect and twenty-six novel viruses were detected. Seven novel viruses showed weakly similarity to honeybee pathogenic viruses and five of them were determined can be replicated their genomes in the corresponding host by detected complementary strands of viral genomes, suggests that they may be pathogenic to the corresponding host.

Conclusion: So many novel viruses detected in wild insect pollinators and their predators indicates that we don't have a clear understanding of the virus composition in pollinator insect and related species. The same living environment provides the conditions for virus cross infection, we should be alert to this situation in the protection of pollinating insects.

Background

Insect pollinators are necessary for most flowering plants, and they play a key role in wild plant reproduction and food security [1, 2]. Honey bee is the most popular pollinator because of its large population and pollination capability. However, other pollinators are also necessary to maintain ecological stability and to increase crop yield. Pollinator biodiversity is critical for pollination quality in agricultural productivity and conservation of the ecosystem [3, 4]. The number of insect pollinators, including bees, is declining worldwide [5, 6]. Several factors underlying pollinator decline can be mainly summarized as follows: heavy use of pesticides; worldwide spread of parasites, especially *Varroa destructor* mites; diseases caused by pathogenic viruses and other pathogens; monoculture cropping and plant biodiversity reduction; and competition between native and invasive species [5, 7, 8]. Adding further complexity to the issue, many of these factors act simultaneously on insect pollinators and can exert additive or even synergistic effects; for example, *V. destructor* is a vector of several honey bee viruses. High-quality food can help honey bee fight viruses. Some insecticides can increase the sensitivity of bees to viruses by affecting their immune mechanism [9–15].

Virus infection is a serious threat to honey bee, which can be infected by at least 25 types of viruses [16, 17]. Of these, seven viruses responsible for severe diseases that threaten beekeeping are the deformed wing virus (DWV), acute bee paralysis virus (ABPV), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), Israel acute paralysis virus (IAPV), Kashmir bee virus (KBV), and Sacbrood virus (SBV) [18].

The application of next-generation sequencing technology in virus discovery has extended our understanding of viruses [19, 20]. More than ten new honey bee pathogenic viruses have been detected by sequencing technology. These include *Apis mellifera* filamentous virus (AMFV), Lake Sinai virus (LSV), *Varroa* orthomyxovirus-1 (VOV-1), *Apis mellifera* bynyavirus-1 and -2, *Apis* dicistrovirus, *Apis mellifera* flavivirus, *Apis mellifera* nora virus-1, *Apis mellifera* rhabdovirus-1 and -2, and Big Sioux River virus [17, 21]. Furthermore, the discovery of new subtypes of known viruses, such as DWV-type B, DWV-type C, and several subtypes of LSVs, depends on sequencing technology [22–24]. Although we have accumulated much genome information on honey bee-related viruses, the evolutionary relationships of some honey bee viruses have not been expounded clearly. CBPV has two genome segments (segment 1: 3674 bp, segment 2: 2305 bp); its special genome structure and putative RNA-dependent RNA polymerase (RdRp) sequence have very low similarity to other viruses. One can only speculate that it may have an association with nodavirus and tombusvirus [25]. LSV was classified as *Nodaviridae*, but the LSV-containing clade was distinct from the *Nodaviridae* family in an unrooted phylogenetic tree, and its several subtypes were detected in honey bee [22, 26].

Virus research on insect pollinators has mainly focused on bees. In addition, compared with other factors threatening insect pollinators such as pesticides and food shortage, viruses are more easily ignored until before causing a disease [27]. Non-*Apis* insect pollinators are necessary for the ecosystem, but their virological investigation is often overlooked [3, 28]. The honey bee pathogenic virus DWV is known to spread widely in wild insect pollinators, and other pathogenic viruses such as BQCV, SBV, and IAPV were detected in non-*Apis* hymenopteran species [29–31]. In this study, we used next-generation sequencing technology to investigate the viruses of non-*Apis* pollinators and their predators, hoping to expand our understanding of the viruses of insect pollinators and to provide some information for the protection of wild insect pollinator diversity.

Materials And Methods

Sample collection and processing

More than 50 species of pollinators and their predators in the field were collected in Xiangshan, Beijing, from August to October 2020. All insects were collected on flowers (insect pollinators) or next to flowering plants (wasp-like insect predators). They were immediately stored in 5-mL centrifuge tubes in an ice box, and transferred in – 80 °C refrigerator until processing. Insects were individually ground into powder in liquid nitrogen and mixed into two pools for subsequent sequencing. Picture of all insect samples can be found in Supplementary Fig. 1 ~ Fig. 4.

Total RNA extraction and sequencing

All insect samples were ground separately in frozen condition and divided into two pools: pool A (pollinators) and pool B (predators). It is worth noting that we cannot determine the feeding habits of insects according to their morphology, and this grouping is preliminary. Sample composition of the pools can be found in Supplementary Table 1. Total RNA of the two mixed pools was extracted using the

RNApure Total RNA Kit (RN0302; Aidlab Biotechnologies Co. Ltd., Beijing, China) according to the manufacturer's protocol. Sequencing libraries were generated using VAHTS mRNA-seq v2 Library Prep Kit for Illumina (NR601-01; Vazyme, Nanjing, China) following the manufacturer's recommendations, and index codes were added to attribute sequences to each sample. The libraries were sequenced on an Illumina NovaSeq platform to generate 150-bp paired-end reads according to the manufacturer's instructions. Finally, we obtained a total of 37 Gb clean reads, data is stored in the SRA database with accesssion: SRR14554108 - SRR14554111.

Sequence assembly and virus detection

Sequencing reads were *de novo* assembled using Trinity v2.12.0 [32]. The contigs were compared to the GenBank NR database by Diamond v2.0.8.146 [33]. We used a python script to filter out non-virus contigs. Virus-like contigs were mapped using Bowtie 2 v2.4.2 [34] to evaluate the contig quantity. Virus ORFs were annotated based on the result of ORFfinder v0.4.3 [35] and the structure of the most closely related viral genome. Conserved domains of new viruses were identified using NCBI CDD BLAST v2.11.0 [36]. We ignored viruses for which the whole genome could not be obtained. Sequences of these viruses were either fragmentary genomes or had very few reads.

Phylogenetic analyses

RdRp regions identified by CDD were aligned between target viruses and related viruses using MUSCLE v3.8.31 [37, 38]. All ambiguously aligned regions were subsequently removed using TrimAl v1.2rev59 [39]. The best-fit model of amino acid substitution in each dataset was determined using ModelTest [40]. Phylogenetic trees were then inferred using the maximum likelihood method implemented in RaxML with 1000 bootstrap replicates [41]. Phylogenetic trees were displayed and annotated using FigTree v1.4.4.

Identification of virus hosts

We extracted RNA from each insect powder using the RNApure Total RNA Kit (Aidlab). cDNA was prepared using TaKaRa PrimeScript™ RT Reagent Kit (Perfect Real Time RR037A; Takara Biomedical Technology Co. Ltd., Beijing, China). The presence of a virus was determined using PCR, which was carried out with the first-strand cDNA products using the 2×TSINGKE Master Mix (blue) (TSE004; Beijing TsingKe Biotech Co., Led., Beijing, China) in 25-μL reactions and specific primers. The specific primers were designed based on the assembled viral genome sequences. PCR products were confirmed by agarose gel electrophoresis and Sanger DNA sequencing. Details of primer information are shown in Supplementary Table 1.

Prediction of potential pathogenicity of novel viruses

We used the reverse complementary strand of the virus to predict the pathogenicity of novel viruses. The detection process and reagents used were the same as those in reverse transcription-polymerase chain reaction (RT-PCR), except for specific primers. Specific primers were designed based on the assembled viral genome sequences, and a tag sequence was added to the forward primer. In reverse transcription, RNA was reverse transcribed with tagged forward primers and the control group with random primers.

Then the tag was used as the forward primer and normal reverse primer for PCR detection of cDNA products. PCR products were also confirmed by agarose gel electrophoresis and Sanger DNA sequencing. Details of primer information are shown in Supplementary Table 1.

Results

Detection of known viruses

To obtain the virus information from the sequencing data, we used the Trinity software to assemble the sequencing data, and then the contigs were annotated using the Diamond program before querying the GenBank non-redundant (NR) database. We detected eight known viruses in the annotated results (Table 1). Three of these were the honey bee pathogenic viruses DWV, ABPV, and CBPV. Mayfield virus 1 was first detected in *Bombus terrestris* in Lebanon and UK, *Vespa velutina* associated acypi-like virus was first detected in *V. velutina nigrithorax* in France, Scaldis River bee virus was first detected in *Osmia cornuta* in Belgium, *Arboretum almendravirus* was first detected in the mosquito *Psorophora albigena* in Peru, and Hubei diptera virus 6 was first detected in Diptera in China. The repeated detection of these viruses in different places and times indicates that they may be prevalent in their corresponding hosts and may exhibit pathogenicity.

Table 1
Known viruses detected in this study

Virus name	Reads number	Closest nucleotide accession	Coverage (%)	Identity (%)
DWV	33345	AB070959.1	100	96.65
ABPV	2598	MN565031.1	99	96.78
CBPV RNA1/RNA2	332	KX168412.1	100	97.88
	228	MF175174.1	95	98.44
Mayfield virus 1	13682	MH614304.1	96	94.94
<i>Vespa velutina</i> associated acypi-like virus	249862	MN565043.1	98	94.64
Scaldis River bee virus	694	KY053857.1	94	74.88
Hubei diptera virus 6 RNA1/RNA2	8923	KX884805.1	99	92.54
	2510	KX884806.1	96	92.32
<i>Arboretum almendravirus</i>	30383	KC994644.1	100	77.56
Note: Number of reads was obtained using Bowtie 2. Reads were mapped to viral genomes using SAMtools stats. The online BLASTn tool was used to compare the assembled viral genome sequences with those in the database.				

Identification of novel RNA viruses

In this study, a total of 26 novel RNA viruses were identified (Table 2). Open reading frames (ORFs) of novel viruses were identified by the ORFfinder software and referred to the genome structure of closely related viruses. Compared with the related viruses, ORFs of all novel viruses were complete or nearly complete. RdRp regions of the novel viruses were checked with the online BLASTp tool, and the identity value with the closest virus was 35.3–71.3%, indicating that these viruses were quite different from known viruses. The phylogenetic tree constructed using the RdRp region showed that 25 novel viruses could be divided into 14 virus families: *Flaviviridae* (2), *Iflaviridae* (5), *Narnaviridae* (1), *Nyamiviridae* (1), *Orthomyxoviridae* (1), *Polycipiviridae* (1), *Rhabdoviridae* (5), *Sinhaliviridae* (1), *Solinviviridae* (1), *Secoviridae* (1), *Tombusviridae* (1), *Totiviridae* (1), *Tymoviridae* (1), and *Virgaviridae* (3). The two unclassified viruses were related to CBPV and Negevirus (Fig. 1 and Table 2).

Table 2
Classification and genome size of novel viruses

Virus name	Order	Closest family	Genome size (bp)	Closest relative (% RdRp amino acid identity)
Xiangshan nege-like virus	Unclassified	Unclassified	8848	Andrena haemorrhoea nege-like virus (68.2)
Xiangshan virga-like virus 1	Martellivirales	<i>Virgaviridae</i>	9205	Hubei virga-like virus 1 (54.6)
Xiangshan virga-like virus 2	Martellivirales	<i>Virgaviridae</i>	9440	Hubei virga-like virus 1 (54.6)
Xiangshan virga-like virus 3	Martellivirales	<i>Virgaviridae</i>	11269	Hubei virga-like virus 15 (53.5)
Xiangshan orthomyxo-like virus	Articulavirales	<i>Orthomyxoviridae</i>	2336 + 2233 + 1994 + 1604 + 1443 + 967	Dhori thogotovirus (subunit PA: 47.3)
Xiangshan tombus-like virus	Tolivirales	<i>Tombusviridae</i>	2261 + 1722	Cushing virus (69)
Xiangshan insect virus	Unclassified	Unclassified	3665 + 2045	Wuhan insect virus 21 (50.7)
Xiangshan noda-like virus	Nodamuvirales	<i>Sinhaliviridae</i>	5770	Lake Sinai virus (51.2)
Xiangshan picorna-like virus 1	Picornavirales	<i>Iflaviridae</i>	9379	Soybean thrips ifla-like virus 10 (61.8)
Xiangshan picorna-like virus 2	Picornavirales	<i>Iflaviridae</i>	10050	PNG bee virus 13 (69.6)
Xiangshan picorna-like virus 3	Picornavirales	<i>Iflaviridae</i>	9087	Iflavirus IricIV-1 (53.4)
Xiangshan picorna-like virus 4	Picornavirales	<i>Iflaviridae</i>	10087	Darwin bee virus 3 (60.7)
Xiangshan picorna-like virus 5	Picornavirales	<i>Iflaviridae</i>	9918	Hubei odonate virus 4 (70.7)

Note: RdRp regions of novel viruses were identified using the Conserved Domain Database (CDD), and the BLASTp online tool was used to search for the closest virus relative. The closest relative of the Xiangshan orthomyxo-like virus was compared using polymerase subunit PA amino acid sequences, and the narna-like virus tree used complete RdRp amino acid sequences.

Virus name	Order	Closest family	Genome size (bp)	Closest relative (% RdRp amino acid identity)
Xiangshan picorna-like virus 6	Picornavirales	<i>Polycipiviridae</i>	11387	Linepithema humile polycipivirus 1 (53.8)
Xiangshan picorna-like virus 7	Picornavirales	<i>Solinviviridae</i>	10200	Diabrotica undecimpunctata virus 1 (39.4)
Xiangshan rhabdo-like virus 1	Mononegavirales	<i>Rhabdoviridae</i>	10798	Menghai rhabdovirus (53.4)
Xiangshan rhabdo-like virus 2	Mononegavirales	<i>Rhabdoviridae</i>	13241	Sanxia Water Strider Virus 5 (49.9)
Xiangshan rhabdo-like virus 3	Mononegavirales	<i>Rhabdoviridae</i>	11774	Hymenopteran rhabdo-related virus OKIAV109 (60.8)
Xiangshan rhabdo-like virus 4	Mononegavirales	<i>Rhabdoviridae</i>	12645	Hymenopteran rhabdo-related virus OKIAV24 (70.4)
Xiangshan rhabdo-like virus 5	Mononegavirales	<i>Rhabdoviridae</i>	11517	Lepidopteran rhabdo-related virus OKIAV3 (60.9)
Xiangshan nyami-like virus	Mononegavirales	<i>Nyamiviridae</i>	9707	Hymenopteran orino-related virus OKIAV85 (72)
Xiangshan narna-like virus	Wolframvirales	<i>Narnaviridae</i>	3191	Wenling narna-like virus 8 (35.3)
Xiangshan tymo-like virus	Tymovirales	<i>Tymoviridae</i>	9087	Nasturtium officinale macula-like virus 1 (71.3)
Xiangshan ghabri-like virus	Ghabrivirales	<i>Totiviridae</i>	6009	Camponotus yamaokai virus (57.3)
Xiangshan flavi-like virus 1	Amarillovirales	<i>Flaviviridae</i>	14732	Shayang fly virus 4 (47)
Xiangshan flavi-like virus 2	Amarillovirales	<i>Flaviviridae</i>	17417	Shayang fly virus 4 (48.6)
Note: RdRp regions of novel viruses were identified using the Conserved Domain Database (CDD), and the BLASTp online tool was used to search for the closest virus relative. The closest relative of the Xiangshan orthomyxo-like virus was compared using polymerase subunit PA amino acid sequences, and the narna-like virus tree used complete RdRp amino acid sequences.				

Seven novel viruses showed a weak relation with honey bee pathogenic viruses. The multiple sequence alignment comparison by log-expectation (MUSCLE) program was used to determine the similarity between novel viruses and related known honey bee viruses. Four novel viruses in the order *Picornavirales* were phylogenetically related to honey bee pathogenic viruses. Xiangshan picorna-like virus (XPLV) 2 and XPLV4 are phylogenetically related to the key honey bee pathogenic virus DWV (NC_004830.2); the amino acid identity values were 45.94% and 46.39%, respectively. XPLV5 is phylogenetically related to the slow bee paralysis virus (SBPV, NC_014137.1); the amino acid identity value was 41.04%. XPLV1 is phylogenetically related to SBV (NC_002066.1); the amino acid identity value was 47.26%. A novel virus in Orthomyxoviridae, Xiangshan orthomyxo-like virus (XOLV), is weakly related to VOV-1; the polymerase subunit PA amino acid identity value was 34.98%. VOV-1 is a new virus recently discovered to infect bees, and it can replicate in both *V. destructor* and *A. mellifera* [17]. Xiangshan insect virus (XIV) and Xiangshan noda-like virus (XNLV) is weakly related to CBPV (NC_010711.1; NC_010712.1) and LSV (NC_032433.1); the complete RdRp amino acid identity values were 35.10% and 38.26%, respectively.

Identification of hosts of novel viruses

Because the pool was mixed with several species before sequencing, the hosts of novel viruses could not be obtained from the sequencing data. We selected seven viruses (i.e., XOLV, XTLV, XPLV2, XIV, XNLV, XPLV1, and XPLV4) to identify their hosts. These viruses were presumed to have a segmented genome or were weakly related to key honey bee viruses (i.e., LSV, CBPV, SBV, and DWV). Primers were designed based on novel viral genomes, and these primers were used to screen all samples before RT-PCR to confirm the sample source of novel viruses. Hosts of the novel viruses are listed in Table 2 and shown in Fig. 2. For segmented genome viruses, we further designed specific primers for each segment to confirm that these segments come from one sample. All fragments showed positive results in the corresponding host samples (Fig. 3).

Table 3

Hosts of seven novel viruses

Virus name	Molecule	Sample number and host	Replicate
Xiangshan orthomyxo-like virus	ssRNA(-)	5: Diptera: <i>Eristalis tenax</i> (Linnaeus, 1758)	+
Xiangshan tombus-like virus	ssRNA(+)	58: Diptera: <i>Episyrphus balteatus</i> De Geer	-
Xiangshan picorna-like virus 2	ssRNA(+)	4: Hymenoptera: <i>Amegilla parhypate</i> lieftinck	+
Xiangshan insect virus	ssRNA(+)	33: Hymenoptera: -	-
Xiangshan noda-like virus	ssRNA(+)	40: Hymenoptera: -	+
Xiangshan picorna-like virus 1	ssRNA(+)	53: Diptera: -	+
Xiangshan picorna-like virus 4	ssRNA(+)	36: Hymenoptera: -	+
Note: Hosts denoted by '-' were not identified to the species level. Positive results of the reverse complementary strand were denoted by '+' and negative results were denoted by '-'.			

Single-stranded RNA (ssRNA) viruses produce reverse complementary strands during replication. Whether an ssRNA virus can replicate in the host is determined by the presence of the reverse complementary strand of the ssRNA virus in the host [42–45]. So, to investigate if a novel virus replicated in the host, we screened for the presence of the positive-sense RNA strand of negative-sense genome viruses and the negative-sense RNA strand of positive-sense genome viruses using RNA-strand sense-specific primer-tagged RT-PCR (Fig. 4). Reverse complementary strands of XOLV, XPLV1, XPLV2, XNLV, and XPLV4 were detected in the corresponding host samples. Specific primer information can be found in Supplementary Table 2.

Discussion

RNA sequencing (RNA-Seq) is a powerful tool that has been used to discover novel RNA viruses and to detect pathogenic viruses [19, 46, 47]. Many studies focus on the viromics of honey bee using sequencing methods, and this has greatly improved our understanding of bee viruses [16, 22, 48–50]. Compared to honey bee, there are few virus studies on non-*Apis* insect pollinators, although their survival is under similar threat as honey bee [6, 31, 51, 52]. By using RNA-Seq technology, we preliminarily explored the viral composition of non-*Apis* pollinators and their predators.

Honey bee pathogenic viruses spread to non-*Apis* wild insect pollinators, and at least seven viruses (DWV, BQCV, SBV, IAPV, ABPV, SBPV, and CBPV) were detected in non-*Apis* insect pollinators. DWV and BQCV

were able to replicate their genomes in bumble bees [29, 31, 45]. DWV, ABPV, and CBPV were detected in our sequencing data, and our samples did not include *A. cerana*, *A. mellifera*, or other *Apis* species, indicating that the three honey bee pathogenic viruses were detected in non-*Apis* wild insect pollinators. Our results were consistent with previous reports. Spread of honey bee viruses to other pollinators should be noticed, especially when bees are used to provide commercial pollination services [53]. There is no doubt that commercial pollination services have created great value for agricultural production [54–56], but improving virus detection is an effective means to prevent the transmission of viruses from commercial insect pollinators to wild insect pollinators.

Five previously reported insect viruses (host in parentheses) were found in our samples. Mayfield virus 1 (*B. terrestris*), *V. velutina* associated acypi-like virus (*V. velutina nigrithorax*), Scaldis River bee virus (*O. cornuta*), Hubei diptera virus 6 (Diptera), and *Arboretum almendravirus* (*P. albigenu*) have been previously reported in other places and times [20, 57–59], and these viruses were also detected in our samples, indicating that they may be prevalent in the corresponding host and may exhibit pathogenicity.

We detected 26 novel RNA viruses by assembling sequencing data. Bee-infecting viruses are primarily positive-sense ssRNA (+ ssRNA) viruses of the order *Picornavirales* [27, 60]. Seven of these novel viruses were classified in the order *Picornavirales*, suggesting that viruses of *Picornavirales* also infect non-*Apis* insect pollinators. Viruses in the families *Rhabdoviridae*, *Flaviviridae*, *Orthomyxoviridae*, and *Sinhaliviridae* can infect honey bee [16, 21]. Novel viruses belonging to these families were detected in this study, suggesting that non-*Apis* insect pollinators and their predators have similar virus classes as honey bee. But the difference is that five rhabdoviruses were detected in this study and only two rhabdoviruses have been reported in honey bee. This clearly infers that rhabdoviruses are more common in non-*Apis* pollinators and predators. *Virgaviridae* consists of plant viruses [61], and three novel viruses from this family were detected in this study. We think that these three viruses may have come from the contamination of plants. Due to the complex living environment of insect pollinators, we cannot exclude novel plant, fungal, and bacterial viruses. Seven novel viruses (i.e., XPLV2, XPLV4, XPLV5, XPLV1, XNLV, XIV, and XOLV) showed amino acid similarity to honey bee pathogenic viruses (i.e., DWV, SBPV, SBV, LSV, CBPV, and VOV-1). Five of these could replicate their genomes in the corresponding host, suggesting that they are pathogenic to the corresponding host. In addition, the hosts of these viruses closely interact with honey bee in the same ecosystem, suggesting that they are a potential threat to bees, just as host honey bees may also spread the virus to non-*Apis* insect pollinators [30].

The decline in insect pollinators is simultaneously driven by several factors and could act synergistically [5, 62]. The interaction of multiple factors makes it more difficult to understand the reasons for the decline in insect pollinators. Some insecticides can exert additive or synergistic effects on virus-induced mortality and replication in honey bees by affecting their immune system [12]. Diet and virus infection are related in honey bee. High-quality diet has the potential to reduce mortality in the face of infection with IAPV [10]. Based on this study, we can see that the threat of the virus to wild pollinator insects may exceed our expectations. Not only the spread of bee virus threatens them, but also the virus carried by them may

pose a greater threaten. virus research on insect pollinators mainly focuses on bees, but research on non-*Apis* virus host species and have same habitat as them is scarce.

Conclusions

In conclusion, the study of insect pollinators virology should expand the scope of species, there is a large gap in viruses in wild pollinators and predators that live in the same environment as honey bees, and corss host transmission of the virus should be noted in the future.

Abbreviations

RT-PCR: reverse transcription PCR; DWV: deformed wing virus; ABPV: acute bee paralysis virus; BQCV: black queen cell virus; CBPV: chronic bee paralysis virus; IAPV: Israel acute paralysis virus; KBV: Kashmir bee virus; SBV: Sacbrood virus; AMFV: *Apis mellifera* filamentous virus; LSV: Lake Sinai virus; RdRp: RNA-dependent RNA polymerase; ORFs: open reading frames; CDD: conserved domain database; SBPV: slow bee paralysis virus; ssRNA: single-stranded RNA; RNA-Seq: RNA sequencing.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors declare that they agreed to publish this paper with the permission of the publishing houses.

Availability of data and material

This ~550 bp sequence of PBNSPaV from grapevine in this study was submitted to GenBank with the accession number MH371356.

Competing interests

The authors declare that they have no competing interests.

Funding information

This work was supported by the Scientific Research Foundation for Ph.D.Programs of Zaozhuang University (21/1020708), and Science and technology Program of Zaozhuang (2020NS20).

Authors' contributions

WL conceived and designed the study. YH and DW performed the experiments. NL analyzed the epidemiological data. NL wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors thank the Zaozhuang University for funding this study.

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Figures

Figure 1

Phylogenetic relationship of novel viruses inferred from conserved RdRp amino acid sequences. RdRp conserved sequences of novel viruses and related viruses in the trees were identified by CDD. Trees were constructed using the maximum likelihood method, and the amino acid substitution model is annotated below each tree. The orthomyxo-like virus tree was based on polymerase subunit PA amino acid sequences, and the narna-like virus tree was based on complete RdRp amino acid sequences.

Figure 2

Hosts of seven novel viruses. Sample number is shown below each host.

Figure 3

Detection of each segment of viral genome with multiple fragments. (a) Xiangshan tombus-like virus segments 1 and 2 correspond to lanes 1 and 2. (b) Xiangshan insect virus segments 1 and 2 correspond to lanes 1 and 2. (c) Xiangshan orthomyxo-like virus segments 1–6 correspond to lanes 1–6. All PCR products were further verified by Sanger DNA sequencing.

Figure 4

Detection of complementary strands of XOLV (lanes 3, 4), XTLV (lanes 5, 6), XPLV1 (lanes 7, 8), XPLV2 (lanes 9, 10), XNLV (lanes 11, 12), XIV (lanes 13, 14), and XPLV4 (lanes 15, 16). Lanes 3, 5, 7, 9, 11, 13, and 15 were the respective controls. PCR products of lanes 4, 8, 10, 12, and 16 were further confirmed by Sanger DNA sequencing. Lane 6 had no obvious target product, and lane 14 had a possible target band (732 bp) but failed in the Sanger DNA sequencing validation.

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