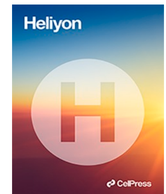
Heliyon 10 (2024) e30186

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Prevalence and species diversity of dsRNA mycoviruses from *Beauveria bassiana* strains in the China's Guniujiang nature

Najie Shi [a,1], Qiuyan Zhu [a,1], Guogen Yang [b], Ping Wang [a], Bo Huang [a,*]

[a] *Anhui Provincial Key Laboratory of Microbial Pest Control, Anhui Agricultural University, Hefei, 230036, China*
[b] *Anhui Province Key Laboratory of Integrated Pest Management on Crops, School of Plant Protection, Anhui Agricultural University, Hefei, 230036, China*

A R T I C L E I N F O

Keywords:
Diversity
dsRNA
Mycoviruses
Beauveria bassiana
Guniujiang nature preserve

A B S T R A C T

We investigated the prevalence and species diversity of dsRNA mycoviruses in *Beauveria bassiana* isolates from the China's Guniujiang Nature Preserve. Among the 28 isolates analyzed, electropherotyping revealed viral infections in 28.6 % (8 out of 28) of the isolates. Metatranscriptomic identification and RT-PCR confirmed the presence of six putative virus species, including two novel species: Beauveria bassiana victorivirus 2 (BbV-2) and Beauveria bassiana bipartite mycovirus 2 (BbBV-2). Four previously characterized mycoviruses were also identified: Beauveria bassiana polymycovirus 4 (BbPmV4), Beauveria bassiana partitivirus 1 (BbPV-1), Beauveria bassiana bipartite mycovirus 1 (BbBV-1), and Beauveria bassiana chrysovirus 2 (BbCV-2). BbPmV4 was found to be the prevailing mycovirus among the infected isolates, and three isolates showed co-infection with both BbPmV4 and BbBV-2. This study enhances our understanding of fungal viral taxonomy and diversity, providing insights into mycovirus infections in *B. bassiana* populations in China's Guniujiang Nature Preserve. Furthermore, the study on the diversity of *B. bassiana* viruses lays the foundation for recognizing fungal viruses as potential enhancers of biocontrol agents.

1. Introduction

Mycoviruses, also known as fungal viruses, are widely present in fungi kingdom. While some mycovirus infections remain hidden, others have been associated with hypovirulence or hypervirulence, leading to significant effects on the growth, development, and reproduction of their fungal hosts [1–4]. The exploration of viral diversity within the virosphere has revolutionized our understanding of virus diversity and evolution [5].

Recent advancements in high-throughput next-generation sequencing (NGS) technologies and bioinformatics have revolutionized our understanding of virus diversity and evolution by enabling the discovery of new viruses across various organisms [5–8]. The advent of high-throughput NGS technologies and metatranscriptomics based on RNA sequencing (RNA-seq) has overcome challenges associated with virus identification and significantly expanded our knowledge of virome and mixed viral infections in single fungal strains [9–13]. Recent studies have increasingly revealed mixed infections of different mycoviruses or dsRNA elements within a single strain, such as in fungal strains *B. bassiana*, *Rosellinia necatrix*, *Botrytis cinerea*, and *Rhizoctonia solani* [9,10,14,15]. For instance,

* Corresponding author.
E-mail address: bhuang@ahau.edu.cn (B. Huang).
[1] Najie Shi and Qiuyan Zhu contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e30186
Received 9 September 2023; Received in revised form 17 April 2024; Accepted 22 April 2024
Available online 26 April 2024
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Rhizoctonia solani strain DC17 was found to be co-infected with 17 different mycoviruses [10], while a *B. bassiana* strain RCEF1446 was found to harbor three mycoviruses [14].

B. bassiana, an entomopathogenic fungus with a broad host range and wide geographical distribution, has gained attention as a potential biocontrol agent against plant pests, leading to its commercial use in many countries [16,17]. Recent studies have identified mycoviruses in *B. bassiana*, highlighting the presence of dsRNA elements, virus-like particles, and their association with hypovirulence and hypervirulence [18–20]. To date, seventeen viruses have been reported in *B. bassiana*, primarily distributed among six known families: *Amalgaviridae* (dsRNA, 1 species), *Totiviridae* (dsRNA, 3 species), *Partitiviridae* (dsRNA, 3 species), *Chrysoviridae* (dsRNA, 2 species), *Polymycoviridae* (dsRNA, 4 species), and *Narnaviridae* (+ssRNA, 1 species). Furthermore, three *B. bassiana* mycoviruses with unique molecular and biological properties, unlike any other known mycoviruses, remain unassigned to established virus families [18]. Mycoviruses from *B. bassiana* have extensively been studied, however, the prevalence and species diversity of dsRNA mycoviruses in *B. bassiana* from the same geographical locations remain largely unexplored.

This study aims to investigate the prevalence and biodiversity of dsRNA mycoviruses in *B. bassiana* strains collected from the Guniujiang nature preserve in China. By analyzing the metatranscriptome of these strains using high-throughput RNA-seq, we identified six mycoviruses in eight out of the twenty-eight *B. bassiana* isolates analyzed. Importantly, two of these mycoviruses represent novel species that have not been previously described, including an unassigned dsRNA mycovirus and a victorivirus. Remarkably, out of the eight infected isolates, three exhibited co-infection with both BbPmV4 and BbBV-2, while the remaining five isolates were singly infected by a mycovirus. The discovery of these mycoviruses not only expands our understanding of mycovirus diversity but also provides valuable insights into virus evolution in fungal isolates originating from the same geographical location.

2. Materials and methods

2.1. Fungus isolation and purification

Strains of *B. bassiana* were collected from the Guniujiang Nature preserve of Anhui province in 2021. The Guniujiang Nature Preserve is composed of five small scenic spots: Main Peak Scenic Spot, Qifeng Scenic Spot, Shuanghekou Scenic Spot, Longmen Scenic Spot and Guanyintang Scenic Spot. We collected 10 samples from each scenic spot, resulting in a total of 28 isolated strains of *B. bassiana*. The identification of *B. bassiana* was based on its morphological characteristics and molecular data, including the sequence of the internal transcribed spacer (ITS) region and the translation elongation factor 1-α (ef1-α) gene. The strains and their derivatives were cultured on sabouraud dextrose agar with yeast extract (SDAY) medium, which consisted of 1 % w/v peptone, 0.2 % w/v yeast extract, 4 % w/v dextrose, and 1.5 % w/v agar. The cultures were maintained at 25 °C under a 12 h light/12 h dark cycle for up to 12 days, unless otherwise specified.

2.2. Total RNA extraction, dsRNA extraction and sequencing

Total RNA was extracted from the fungal isolates using the TRIzol reagent (Invitrogen). The dsRNA fraction was isolated using CF-11 cellulose chromatography (Sigma), as previously described [21]. To remove DNA and single-stranded RNA contaminants, the dsRNA samples were purified through digestion with DNase I and S1 nuclease (TaKaRa, Dalian, China). For Illumina pair-end sequencing, a minimum of 3 μg dsRNA from each sample was employed for library construction. The dsRNA underwent reverse transcription into cDNA. Paired-end libraries with insert sizes of approximately ~450 bp were prepared following Illumina's standard genomic DNA library preparation procedure. The process involved shearing purified genomic DNA into smaller fragments with the desired size using Covaris, and generating blunt ends with T4 DNA polymerase. Following the addition of an 'A' base to the 3′ end of the blunt phosphorylated DNA fragments, adapters were ligated to the ends of the DNA fragments. Subsequently, the desired fragments were purified through gel electrophoresis, selectively enriched, and amplified by PCR. At the PCR stage, the index tag could be introduced into the adapter as appropriate, followed by a library quality test. Finally, the quantified Illumina pair-end library was utilized for sequencing on the HiSeq 2500 platform at BGI (Shenzhen, China).

2.3. Bioinformatics analyses

The clean reads obtained from the sequencing were assembled using a metagenomic *de novo* assembly approach in the CLC Genomics Workbench (version: 6.0.4). The assembled contigs were then subjected to BLAST searches against the non-redundant database using BLASTx to identify homologous viral sequences (Table S1). To generate viral fragments, contigs corresponding to the same viruses were assembled using DNAMAN 7.0 (Lynnon Biosoft, USA) software.

2.4. Confirmation of mycoviruses and full-length determination

To confirm the presence of putative mycoviruses in the strains, cDNA was synthesized using Moloney murine leukemia virus transcriptase (M-MLV, Takara Dalian, China) and hexanucleotide random primers (Takara Dalian, China). Specific primers were designed based on the assembled contigs to amplify regions corresponding to individual mycoviruses. The amplified products were analyzed by agarose gel electrophoresis, cloned into pMD18-T vector (TaKaRa, Dalian, China), and then sequenced. The primers used for RT-PCR analyses are listed in Table S2.

To determine the full-length sequences of the obtained viral genomes, the 5′ and 3′ ends of the dsRNA elements were determined

using RNA-ligase-mediated rapid amplification of cDNA ends (RLM-RACE). The expected PCR amplicons were purified, cloned into pMD18-T vector (Takara), and sequenced. Each base was sequenced in at least three independent clones to ensure sequence accuracy. The primers used for RACE analyses are listed in Table S3.

2.5. Mycovirus genome characteristics and phylogenetic analysis

Homology searches were performed using the BLAST program in NCBI (BLASTp or BLASTx). Potential open reading frames (ORFs) were predicted using the NCBI ORF Finder tool (https://www.ncbi.nlm.nih.gov/orffinder). The potential secondary structures of the viral terminal sequences were predicted using an online tool (http://rna.urmc.rochester.edu/RNAstructure.html). Multiple sequence alignment was performed using MAFFT [22], and a phylogenetic tree was constructed using MEGA X [23]. The tree files were exported to FigTree-1.4.0 for visualization.

3. Results

3.1. Electropherotyping and metatranscriptomic identification of mycoviruses infecting *B. bassiana* isolates

In 2021, a total of 28 strains of *B. bassiana* were collected from the Guniujiang nature preserve and identified based on both morphological features and molecular data. The presence of viral dsRNA molecules was screened in these 28 isolates, and eight isolates (RCEF6857, RCEF6854, RCEF6899, RCEF6861, RCEF6864, RCEF6926, RCEF6869, and RCEF6909) showed distinct dsRNA banding patterns, including a band ranging from approximately 0.7 to 6.0 kb (Fig. 1a). Thus, we detected dsRNA elements indicative of viral infections in 28.6 % of the isolates.

High-throughput sequencing libraries were constructed, and Illumina MiSeq 2500 platform was used to generate approximately 3.6–4.7 $\times$ 10^7 paired-end reads, each with a length of 300 nt. Raw reads underwent cleaning and *de novo* assembly using Trinity, resulting in 19,539 contigs. These contigs were then compared to the *B. bassiana* reference genome, and subsequent BLAST searches against the NCBI viral database identified several contigs as partial genomic segments of distinct mycoviruses. Among them, six contigs showed significant similarity to the amino acid sequences of RNA-dependent RNA polymerase (RdRp) domains from six different viral sequences, suggesting the presence of six distinct, nearly complete mycovirus-like genome sequences.

3.2. The six viruses hosted by *B. bassiana* isolates

To confirm the presence of viruses in the eight *B. bassiana* isolates selected for RNA analysis, RT-PCR was performed using specific primers designed based on the viral contigs (Fig. 1b). The predicted amino acid sequences of the putative viral genomes exhibited significant sequence identity with previously described viruses from different lineages, including the families *Polymycoviridae*,

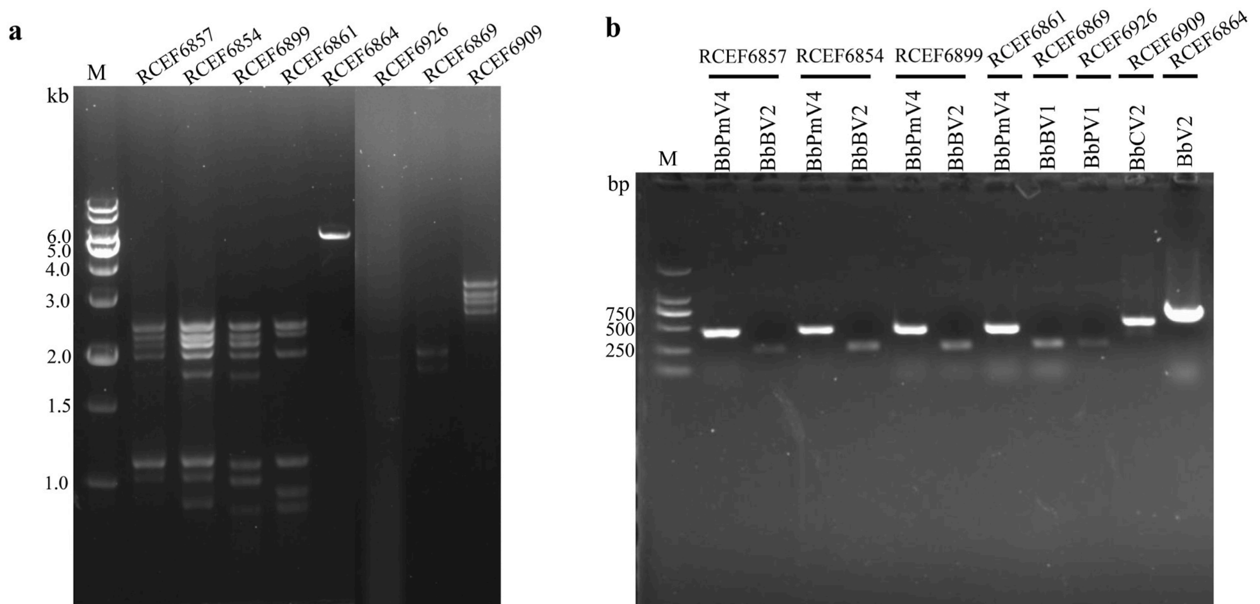

Fig. 1. Double-stranded RNA in *B. bassiana* isolates. (a) 1.5 % (w/v) agarose gel electrophoresis of purified dsRNA fragments from eight *B. bassiana* isolates; Lane M, DNA marker (10000-bp ladder, Dongsheng Biotech). And the original image of the gel is in Fig. S1. (b) RT-PCR analysis of the eight fungal strains. And the original image of the gel is in Fig. S2. Total RNA was isolated from the strains and subjected to RT-PCR using primer sets shown in Supplementary Table S2.

Totiviridae, *Partiviridae*, and *Chrysoviridae*, as well as an unassigned dsRNA mycovirus (Table S1). Notably, two of these assembled viral sequences shared less than 85.61 % amino acid identity with previously described mycoviruses, indicating their novelty.

3.3. One predicted novel virus in family Totiviridae

A contig sequence (contig number 29854) showed 85.61 % nucleic acid sequence identity with members of the genus *Victorivirus* within the family *Totiviridae*. This contig corresponded to an undivided dsRNA genome of a victorivirus named Beauveria bassiana victorivirus 2 (BbV2) in the RCEF6864 strain (Fig. 2a). The complete genome sequence of BbV2 (dsRNA1) was 5,233 bp in length with a GC content of 56.2 %. It possessed two large open reading frames (ORF1 and ORF2), encoding putative coat protein (742 aa, 78.55 kDa) and RdRp (834 aa, 90.96 kDa), respectively (Fig. 2b). The 5′- and 3′-untranslated regions (UTRs) were 448 bp and 52 bp in length, respectively (Fig. 2b). The overlap between the stop codon of ORF1 and the start codon of ORF2 created the pentamer sequences UAAUG (the potential re-initiation start codon is indicated by underlining), and a −1 frameshift was expected between ORF1 and ORF2 (Fig. 2b). The complete sequences of dsRNA1 were assembled using DNAMAN 7.0 (Lynnon Biosoft, USA) and deposited in the GenBank database with accession numbers OR126353. The putative RdRp encoded by ORF2 showed high similarity to the RdRps in

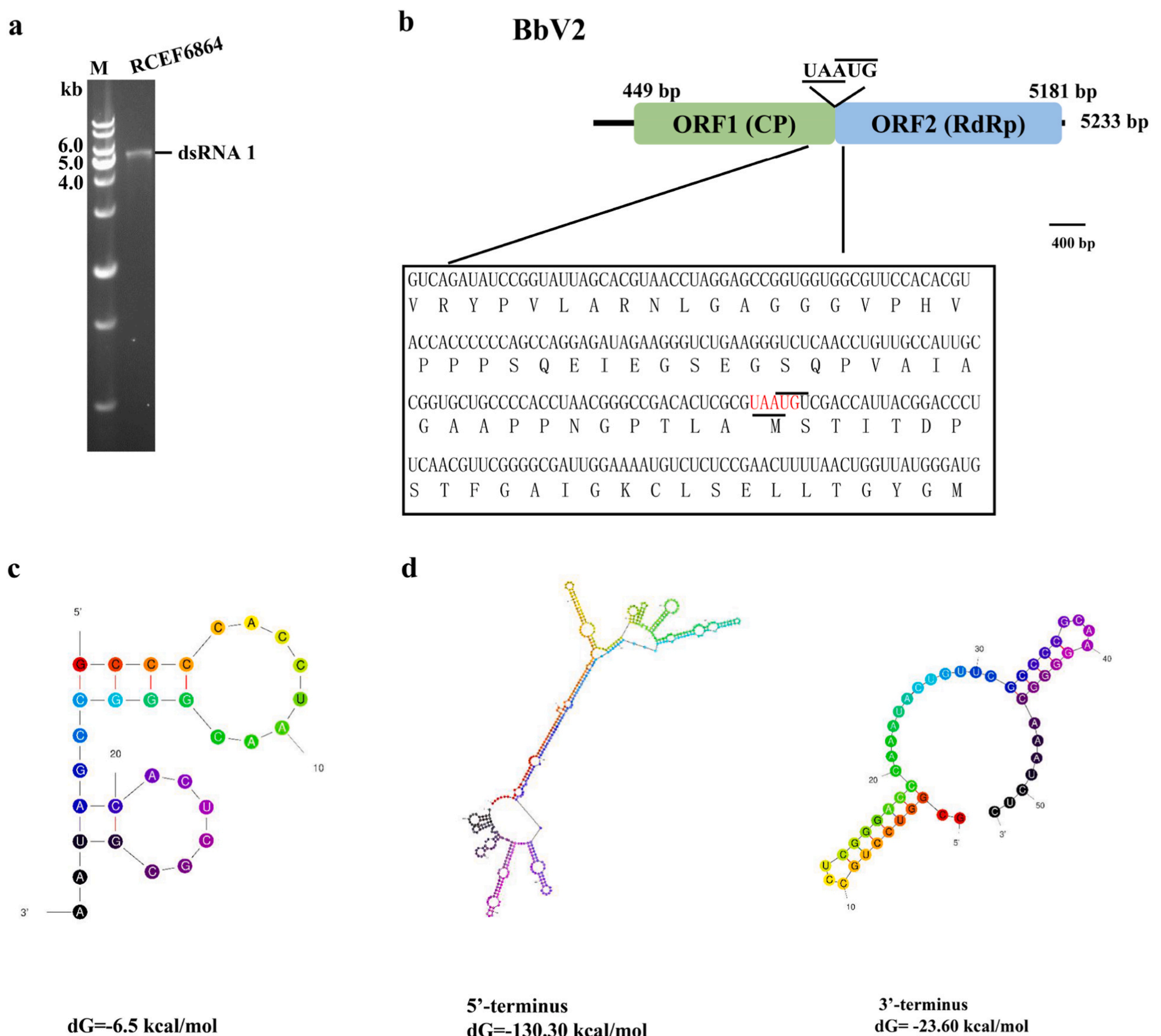

Fig. 2. Analysis of the dsRNA profile and genomic organization of BbV-2. (a) Purified dsRNA extracted from RCEF6864 was electrophoresed in a 1.5 % agarose gel. M, DNA marker (10,000-bp ladder, Dongsheng Biotech). And the original image of the gel is in Fig. S3. (b) Schematic representation of BbV-2, with the amino acid sequence of the C-terminal region of ORF1 showing the Ala/Gly/Pro-rich stretch. (c) Schematic representation of the predicted H-type RNA pseudoknot 30 nt directly upstream of the AUGA motif (underlined) in BbV-2. (d) The secondary structure of the 5′-terminus and 3′-terminus of BbV-2.

the genus *Victorivirus*, including Beauveria bassiana victorivirus 1 (BbV1) (85.61 % aa identity), Cordyceps chanhua victorivirus 1 (69.43 % aa identity), Metarhizium anisopliae M5 victorivirus 1 (55.70 % aa identity) Ustilaginoidea virens RNA virus 1 (53.61 % aa identity) and Ustilaginoidea virens RNA virus 13 (54.06 % aa identity). Similarly, the BLASTp search on NCBI revealed that the putative coat protein encoded by ORF1 showed homology to the coat proteins of victoriviruses and other viruses in the family *Totiviridae*, particularly Beauveria bassiana victorivirus 1 (BbV1) (89.04 % aa identity), Cordyceps chanhua victorivirus 1 (73.51 % aa identity), Metarhizium anisopliae M5 victorivirus 1 (66.67 % aa identity), Ustilaginoidea virens RNA virus 15 (64.18 % aa identity) and Ustilaginoidea virens RNA virus L (63.14 % aa identity).

Additionally, the C-terminal sequence of the virus coat protein was found to be rich in Ala/Gly/Pro amino acids (aa) (Fig. 2b). An H-type pseudoknot structure was predicted upstream of the UAAUG motif of BbV2 at nucleic acid positions 2648–2677 with a ΔG value of −6.5 kcal/mol (Fig. 2c). A predicted pseudoknot structure, which plays a crucial role in stop/re-initiation translation, is anticipated to form immediately preceding the tetranucleotide sequence 5′- AUGA -3’. This structural arrangement occurs at a position analogous to that observed in Hv190SV [24]. Notably, certain victoriviruses and hypoviruses, acting as potential facilitators of stop/restart translation, exhibit a pentanucleotide sequence at their junction, namely 5′-UAAUG-3’. Furthermore, stable secondary structures were predicted in the 5′ and 3′-UTRs of the genome (Fig. 2d). Similar structures have been observed in other victoriviruses, and they are known to be necessary and sufficient for the reinitiation of viral RdRp translation.

	I		II		III		IV	
BbV2	ISGR	(56)	WTSRWLWCVNGS	(50)	KLEHGKTRVILACDTRSY	(44)	NLMLDYDDFNSHH	(45)
CcV1	LAGR	(56)	WTSRWLWCVNGS	(50)	KLEHGKTRAIFACDTRSY	(44)	NLMLDYDDFNSHH	(45)
BbRV1	MAGR	(56)	WSSRWLWCVNGS	(50)	KLENGKTRAIFACDTRSY	(44)	NLMLDYDDFNSHH	(45)
FuToV5	LAGR	(56)	WTSRWLWCVNGS	(50)	KLEAGKTRAIFACDTRSY	(44)	NLMLDYDDFNSQH	(45)
FuToV4	LAGR	(56)	WTSRWLWCVNGS	(50)	KLETGKTRAIFACDTRSY	(44)	NLMLDYDDFNSQH	(45)
FuToV3	LAGR	(56)	WSRRWLWCVNGS	(50)	KLEHGKTRAIFACDTRSY	(44)	NLMLDYDDFNSQH	(45)
FTV2	LAGR	(56)	WTSRWAWCVNGS	(50)	KLEHGKTRAIFSCDTRSY	(44)	NLMLDYDDFNSQH	(45)
UvRV1	LAGR	(56)	WSARWLWCVNGS	(50)	KLEHGKTRAIFSCDTRSY	(44)	NLMLDYDDFNSQH	(46)
UvRV15	LAGR	(56)	WSRRWLWCVNGS	(50)	KLEHGKTRAIYACDTRSY	(44)	NLMLDYDDFNSHH	(45)
AfV-S1	LQGR	(56)	WTSRWAWCVNGS	(50)	KLENGKERAIFACDTRSY	(44)	NLMLDYDDFNSHH	(45)
FsamVV1	LQGR	(56)	WSARWLWCVNGS	(50)	KLENGKERAIFACDTNSY	(44)	NLMLDYDDFNSQH	(45)
FsamVV2	LQGR	(57)	WTSRWAWCVNGS	(50)	KLEAGKTRAIFACDTRSY	(44)	NLMLDFDDFNSQH	(45)
CcVV1	LQGR	(56)	WSSRWAWCVNGA	(49)	KLEHGKTRAIFACDTRSY	(44)	NLMLDFDDFNSHH	(45)
SnVV1	LQGR	(56)	WTSRWAWCVNGS	(49)	KIEHGKTRAIFACDTRSY	(44)	NLMLDFDDFNSHH	(45)
	**		* ** ******		* * ** * * *** **		***** **** *	

	V		VI		VII		VIII
BbV2	GTLMSGHRATTFINSVLNAAYIR	(12)	SLHTGDDVYIR	(17)	GCRMNPAKQSIG	(3)	AEFLR
CcV1	GTLMSGHRATTFINSVLNAAYIR	(12)	SLHTGDDVYIR	(17)	GCRMNPAKQSIG	(3)	AEFLR
BbRV1	GTLMSGHRGTTFINSVLNAVYIR	(12)	SLHTGDDVYVR	(17)	GCRMNPAKQSIG	(3)	AEFLR
FuToV5	GTLMSGHRGTTFINSVLNAAYIR	(12)	SLHTGDDVYIR	(17)	GCRMNPTKQSIG	(3)	AEFLR
FuToV4	GTLMSGHRGTTFINSVLNAAYIR	(12)	SLHTGDDVYIR	(17)	GCRMNPTKQSIG	(3)	AEFLR
FuToV3	GTLMSGHRATTFINSVLNAAYVR	(12)	SLHAGDDVFFR	(17)	GCRMNPTKQSIG	(3)	AEFLR
FTV2	GTLMSGHRATTIINSVLNAAYIR	(12)	SLHTGDDVFFR	(17)	GCRLNPTKQSIG	(3)	AEFLR
UvRV1	GSLMSGHRGTTFVNSVLNAAYIR	(12)	SLHTGDDVYMR	(17)	GCRMNPTKQSVG	(3)	AEFLR
UvRV15	GTLMSGHRATTFINSVLNAAYIR	(12)	SLHAGDDVYVR	(17)	GCRMNPTKQSIG	(3)	AEFLR
AfV-S1	GTLMSGHRGTTFFNSILNAAYVR	(12)	SLHAGDDVYIR	(17)	GCRMNPTKQSIG	(3)	AEFLR
FsamVV1	GTLMSGHRGTTFFNSVLNAAYIR	(12)	SLHAGDDVYIR	(17)	GCRMNPSKQSIG	(3)	AEFLR
FsamVV2	GTLMSGHRGTTFINSVLNAAYIR	(12)	SLHTGDDVYIR	(17)	GCRLNPAKQSVG	(3)	AEFLR
CcVV1	GTLMSGHRGTTFFNSVLNAAYFR	(12)	SLHTGDDVYAR	(17)	GCRLNPTKQSIG	(3)	AEFLR
SnVV1	GTLMSGHRGTTFFNSILNAAYFR	(12)	SLHTGDDVYAR	(17)	GCRLNPTKQSIG	(3)	AEFLR
	* ****** ** ** ***** *		*** **** *		*** ** *** *		*****

Fig. 3. Conserved amino acid sequences in the RdRps of *Victorivirus*. Identical residues are indicated by the same background color and an asterisk. The abbreviations for the viruses are presented in Supplementary Table S4. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Recent reports recommend more stringent values of pairwise percent identity for species delimitation within the genus *Victorivirus*: 80 % for CP sequences and 90 % for RdRp sequences, in addition to fungal host identity [25]. Furthermore, a maximum likelihood (ML) phylogenetic tree was generated employing the LG + G + I + F model, utilizing the complete amino acid sequence of the RdRp (ORF2) from BbV2 and selected members of the *Totiviridae* family. The analysis revealed that BbV2 forms a distinct clade with previously reported victoriviruses (Fig. S5). Additionally, sequence analysis employing the conserved domain database (CDD) and a multiple sequence alignment demonstrated that the RdRp sequences of BbV2 possess a conserved viral RdRp domain (RdRp_4; pfam02123) featuring eight motifs (I–VIII), characteristic of conserved elements in dsRNA viruses. Notably, these motifs include the GDD motif, which is a characteristic of mycoviral RdRps (Fig. 3) [26].

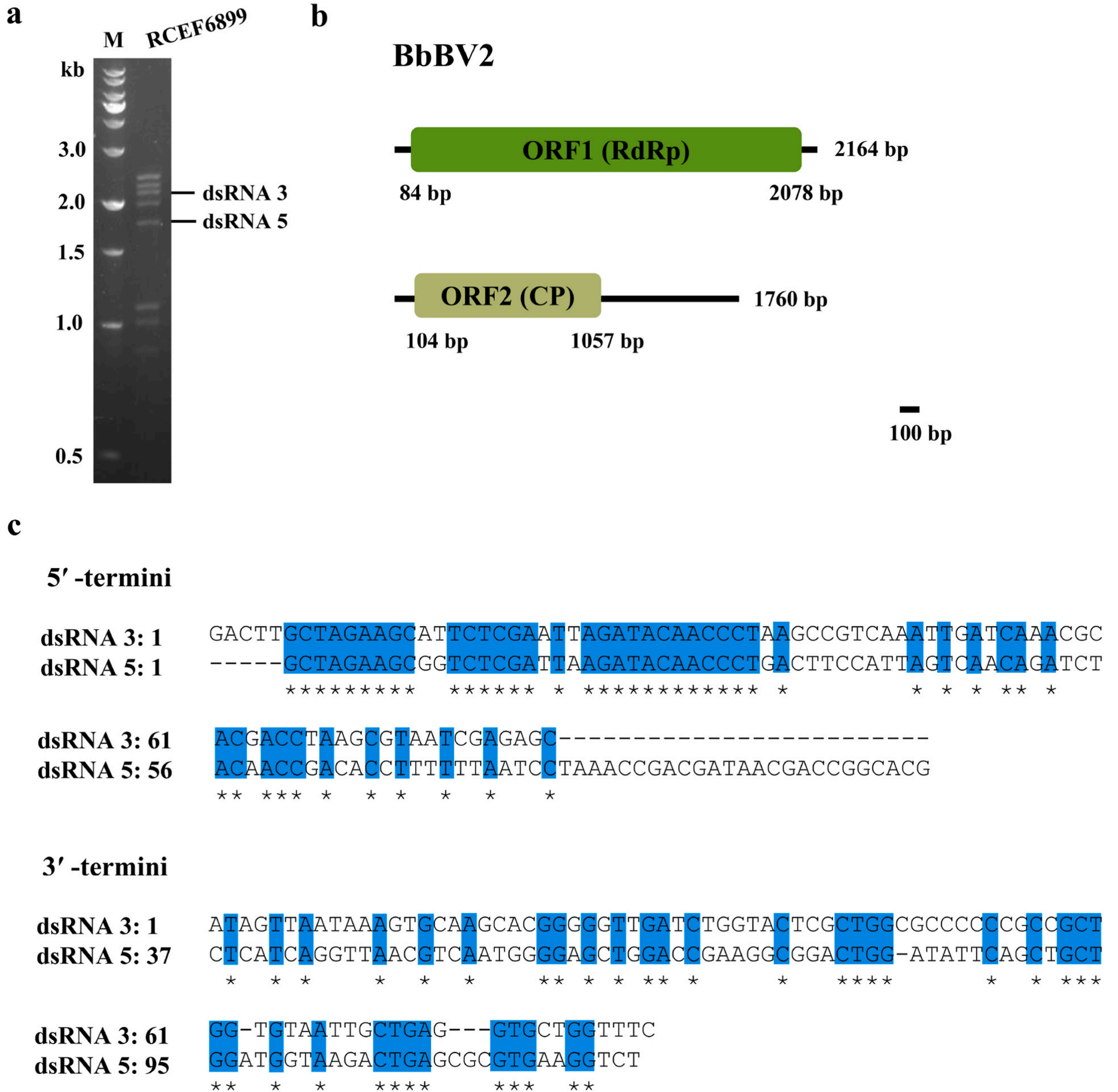

Fig. 4. Analysis of the dsRNA profile and genomic organization of the novel mycovirus (BbBV2) from *B. bassiana* strain RCEF6899. And the original image of the gel is in Fig. S4. (a) Purified dsRNA extracted from *B. bassiana* strain RCEF6899 was electrophoresed in a 1.5 % agarose gel. M, DNA marker. (b) Schematic representation of the BbBV2 genomic structure. (c) Comparison of the 5′- and 3′-terminal sequences of dsRNA 3 and dsRNA 5 of BbBV2. "*" indicates a conserved nucleotide.

3.4. A novel bipartite mycovirus

Two viral contig sequences (CL1861.Contig1 and Unigene34295) showed similarity to Fusarium graminearum dsRNA mycovirus 4 (Fgv4) and Fusarium graminearum dsRNA mycovirus 5 (Fgv5). These contigs were designated as BbBV2. Fgv4 and Fgv5 are unassigned dsRNA viruses consisting of two dsRNA segments of approximately 2 kb, encoding a putative viral RNA polymerase and a putative protein. Analysis revealed that BbBV2 contains two dsRNA segments (dsRNA 3 and dsRNA 5) in RECF6899 (Fig. 4a), RECF6854, and RECF6857, and the genomic organization is depicted in Fig. 4b.

dsRNA 3 and dsRNA 5 had G + C contents of 55.3 % and 58.0 %, respectively, and lacked a poly(A) tail. Further analysis indicated that dsRNA 3 (2164 nt) contained a single open reading frame (ORF1; nt 84–2078), which encoded a putative RdRp comprising 664 aa with a mass of approximately 76.22 kDa (Fig. 4b). Similarly, dsRNA 5 harbored a single large ORF (ORF 2; nt 104–1057), which encoded a hypothetical protein of 331 aa with a molecular mass of 35.27 kDa (Fig. 4b). The 5′-UTRs of dsRNA 3 and dsRNA 5 were 83 and 103 nt in length, respectively, while the corresponding 3′-UTRs were 86 and 703 nt in length (Fig. 4b).

Sequence alignment of the two 5′-UTRs revealed a highly conserved element (GCTAGAAGCNNTCTCGANTNAGATACAACCCT) that may be essential for virus replication. Although the 3′-UTRs of the two dsRNAs differed significantly in length, some conserved sequences were identified (Fig. 4c). The presence of an extended 3′-UTR on dsRNA 5, which is sometimes polyadenylated, appears to be a diagnostic signature of this group of bipartite mycoviruses. The complete sequences of dsRNA 3 and dsRNA 5 were assembled using DNAMAN 7.0 (Lynnon Biosoft, USA) and deposited in the GenBank database with accession numbers ON938188 and ON938189. Utilizing a phylogenetic tree constructed through the ML method with the LG + G + I model, it is proposed that the virus BbBV2 represents a novel member of the unassigned virus group (Fig. S6). Further analysis through multiple alignments unveiled that the RdRp domain of BbBV2 encompasses eight conserved motifs in the C-terminus of the ORF1-encoded protein, a feature commonly observed in dsRNA viruses (Fig. 5).

3.5. Species diversity of dsRNA mycoviruses from the B. bassiana in the Guniujiang nature preserve

We observed that 8 out of 28 *B. bassiana* isolates collected from the Guniujiang Nature preserve were found to harbor dsRNA elements. Among the isolates, the strains RCEF6861, RCEF6857, RCEF6854, and RCEF6899 contained the virus Beauveria bassiana polymycoviruses 4 (BbPmV4) from the family *Polymycoviridae* (Table 1). Thus, the most prevalent mycovirus was BbPmV4 in these infected strains. Additionally, the electrophoretic patterns and sizes of the dsRNAs observed in Fig. 1a indicated that RCEF6857, RCEF6854, and RCEF6899 harbored both BbPmV4 and BbBV2. The remaining five strains each contained a single distinct fungal virus.

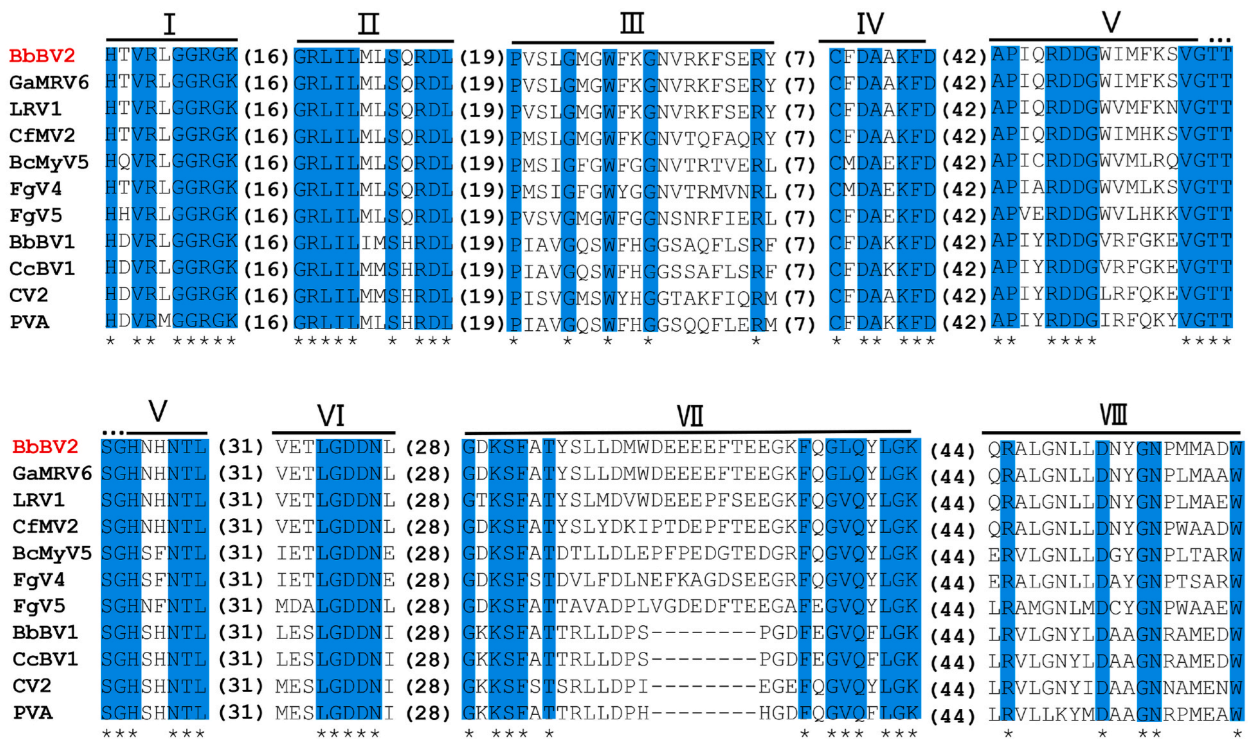

Fig. 5. Conserved amino acid sequences in the RdRps of bipatite mycoviruses. Identical residues are indicated by the same background color and an asterisk. The abbreviations for the viruses are presented in Supplementary Table S4. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Mycovirus-infected *B. bassiana* isolates, their habitat and location.

Species	Isolate	Habitat	Location	Mycovirus[a]
B. bassiana	RCEF6857	*Apoidea*	Guniujiang scenic area	BbPmV4+BbBV2
B. bassiana	RCEF6854	*Cantharidae*	Guniujiang scenic area	BbPmV4+BbBV2
B. bassiana	RCEF6899	*Curculionidae*	Guniujiang scenic area	BbPmV4+BbBV2
B. bassiana	RCEF6861	*Chrysomelidae*	Guniujiang scenic area	BbPmV4
B. bassiana	RCEF6864	*Chrysomelidae*	Guniujiang scenic area	BbV2
B. bassiana	RCEF6926	*Chrysomelidae*	Guniujiang scenic area	BbPV1
B. bassiana	RCEF6869	*Chrysomelidae*	Guniujiang scenic area	BbBV1
B. bassiana	RCEF6909	*Buprestidae*	Guniujiang scenic area	BbCV2

[a] BbPmV4: Beauveria bassiana polymycovirus 4; BbBV1: Beauveria bassiana bipartite mycovirus 1; BbBV2: Beauveria bassiana bipartite mycovirus 2; BbV2: Beauveria bassiana victorivirus 2; BbPV1: Beauveria bassiana partitivirus 1; BbCV2: Beauveria bassiana Chrysovirus 2.

For instance, RCEF6864 harbored a large dsRNA element of 5,233 bp, which was identified as a victorivirus. RCEF6926 and RCEF6869 strains contained two dsRNA elements each, with RCEF6926 harboring Beauveria bassiana partitivirus 1 (BbPV1) and RCEF6869 containing Beauveria bassiana bipartite mycovirus 1 (BbBV1). Additionally, RCEF6909 and RCEF6861 exhibited four dsRNA elements of BbCV2 and four dsRNA elements of BbPmV4, respectively. These findings indicate that the mycoviruses present in *B. bassiana* exhibit significant diversity, even among isolates originating from the same geographical location.

4. Discussion

In this study, through the analysis of 28 isolates from the Guniujiang Nature Preserve, viral infections were detected in 28.6 % of the samples. Using advanced techniques, six putative virus species were identified, including two novel species (Beauveria bassiana victorivirus 2 and Beauveria bassiana bipartite mycovirus 2) and four previously known mycoviruses. The prevailing mycovirus among the infected isolates was Beauveria bassiana polymycovirus 4, with co-infection observed in three isolates.

It is worth noting that the identified mycoviruses in this study belong to different families, including *Polymycoviridae*, *Totiviridae*, *Partitiviridae*, and *Chrysoviridae*, as well as an unassigned dsRNA mycovirus. This indicates that *B. bassiana* isolates from the Guniujiang Nature preserve host a diverse range of mycoviruses with distinct genomic characteristics and evolutionary origins. Furthermore, the discovery of a novel virus, Beauveria bassiana victorivirus 2 (BbV2), belonging to the family *Totiviridae*, adds to the growing knowledge of mycovirus diversity. BbV2 exhibits unique genomic features, including the presence of two large open reading frames (ORFs) encoding a putative capsid protein (CP) and an RdRp. The presence of an H-type pseudoknot structure upstream of the UAAUG motif and stable secondary structures in the 5′ and 3′-UTRs suggests functional significance in viral replication and translation initiation [27–29].

In addition to BbV2, the identification of a bipartite mycovirus, Beauveria bassiana bipartite mycovirus 2 (BbBV2), in several *B. bassiana* isolates is noteworthy. BbBV2 consists of two dsRNA segments encoding putative viral RNA polymerase and protein, similar to Fusarium graminearum dsRNA mycoviruses 4 and 5 (Fgv4 and Fgv5) [30,31]. The conserved elements in the 5′-UTRs and the extended 3′-UTRs on dsRNA 5 provide diagnostic signatures of this group of bipartite mycoviruses. Phylogenetic analysis suggests that BbBV2 represents a novel member of the unassigned virus group.

The presence of multiple mycoviruses in a single strain and the occurrence of different viruses in different strains highlight the complexity of viral interactions within *B. bassiana* populations [19,21]. Co-infections of different mycoviruses or dsRNA elements in a single fungal strain can provide opportunities for virus recombination or horizontal gene transfer, influencing virus evolution, classification, and interactions [20,32–35]. Thus, co-infection and the exchange of genetic material between viruses can have significant implications for the stability and evolution of mycovirus populations [36]. Further research is needed to investigate the dynamics of these interactions and their impact on the biology and pathogenicity of *B. bassiana*.

Although the present study focused on identifying and characterizing mycoviruses in *B. bassiana*, the functional implications of these viral infections were not assessed. Nevertheless, considering the high prevalence of virus infections observed in our study, it is reasonable to expect that some antagonistic associations may exist between the mycoviruses and their *B. bassiana* hosts, as is commonly observed in other known fungus-virus interactions [1,18]. The high-throughput sequencing approach used in this study, coupled with metatranscriptomic analysis, proved to be an effective method for the identification and characterization of mycoviruses in *B. bassiana* isolates. The use of specific primers and RT-PCR confirmed the presence of the putative viral genomes in the selected isolates, further validating the sequencing results (Fig. 1b). This integrated approach allowed for a comprehensive analysis of the viral diversity within the fungal population.

In conclusion, our study reveals the presence of diverse mycoviruses in *B. bassiana* isolates from the Guniujiang Nature preserve. The identification of novel viruses expands our understanding of fungal viral taxonomy and diversity. These findings contribute to the growing body of knowledge on mycovirus-host interactions and provide a foundation for future studies on the potential applications of mycoviruses in biological control strategies. To enhance the insecticidal activity of insect pathogens, our approach involves obtaining single virus-infected strains and virus-free strains through virus elimination or transmission. We then conduct fungal virulence assays on insects to verify whether the virus has a promoting effect on the insecticidal ability of the host.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 32172473).

Data availability statement

The complete sequences of dsRNA 3 and dsRNA 5 for BbBV2 were deposited in the GenBank database with accession numbers ON938188 and ON938189. The complete sequence of dsRNA1 for BbV2 was deposited in the GenBank database with accession numbers OR126353.

CRediT authorship contribution statement

Najie Shi: Writing – original draft. **Qiuyan Zhu:** Methodology. **Guogen Yang:** Supervision. **Ping Wang:** Supervision. **Bo Huang:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30186.

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