Molecular Characterization of A Novel Victorivirus Infecting *Corynespora Cassiicola*

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Abstract

One victorivirus was detected in the isolate of *Corynespora cassiicola* strains 20180909-03, which was named Corynespora cassiicola victorivirus 1 (CcVV1). The whole-genome sequence of the virus was sequenced and identified. The CcVV1 genome is 5140 nt and contains 56.87%GC with two large open reading frames (ORFs) overlapping at the tetranucleotide AUGA. The two ORFs were predicted to encode coat protein (CP) and RNA-dependent RNA polymerase (RdRp) respectively, which were conservative in dsRNA fungal viruses of the family *Totiviridae*. Conservative domains comparison and phylogenetic analysis of the deduced amino acid sequence of RdRp and CP showed that CcVV1 was a new virus of the *Victorivirus* genus. As far as we know, it is the first report of a genomic sequence of the genus *Victorivirus* infecting *Corynespora cassiicola*.

Introduction

Corynespora cassiicola, which is an Ascomycetes fungus, is a necrotrophic plant pathogen and causes a disease commonly known as target spot disease[1]. Severe outbreaks of Corynespora leaf spot disease in many crops, including sesame (*Sesamum indicum*)[2], sweet pepper (*Capsicum annuum*)[3], cotton (*Gossypium hirsutum*)[4], soybean (*Glycine max*)[5] and various economic crops, have had a significant impact on crop production [6]. It has also been found in rare human infections [7].

Mycoviruses are omnipresent and detected in major groups of fungi such as Ascomycota, Basidiomycota, and Glomeromycota [8]. Mycoviruses have diverse genomes including dsDNA, +ssRNA, dsRNA, -ssRNA as their genetic material and have increasingly been reported [9]. Mycoviruses with dsRNA genomes include seven families: *Partitiviridae*, *Reoviridae*, *Totiviridae*, *Megabirnaviridae*, *Chrysoviridae*, *Quadriviridae* and *Endornaviridae* (https://talk.ictvonline.org/taxonomy/). The family *Totiviridae* consists of five genera: *Totivirus*, *Victorivirus*, *Giardiavirus*, *Trichomonasvirus*, and *Leishmaniavirus*. In the *Totiviridae* family, *Totivirus* and *Victorivirus* exclusively infect with fungus [10]. The viruses of the genus *Victorivirus* infect various phytopathogenic fungi, including *Ustilaginoidea virens* [11], *Nigrospora oryzae* [12], *Fusarium asiaticum* [13], *Aspergillus foetidus* [14], *Macrophomina phaseolina* [15] and *Rosellinia necatrix* [16]. The coding strands of victoriviruses usually have two large open reading frames (ORFs) with the 5'-proximal ORF encoding the coat protein (CP) and the 3'-proximal ORF encoding the RNA-dependent RNA polymerase (RdRp) [17]. The stop codon of the upstream CP overlaps with the initiation codon of the RdRp in the tetranucleotide sequence AUGA, which is the feature of the genus *Victorivirus* [10]. The upstream of the AUGA motif usually has an H-type pseudoknot which is determined to be necessary and sufficient for the reinitiation of viral RdRp translation [18]. The CPs of *Victorivirus* have a unique feature among members of the *Totiviridae* family that has an Ala / Gly / Pro enrichment region near its C-terminal [15].

However, Fungal viruses that infect *C. cassiicola* are rarely reported. So far, only one unassigned dsRNA mycoviruse infecting *C. cassiicola* has been reported [19]. As far as we know, this is the first study on

the *Victorivirus* genus infected with *C. cassiicola*. Its genomic organization was determined, and phylogenetic analysis was carried out to elucidate the phylogenetic relationship with other known viruses.

Source Of Virus Material

C. cassiicola strain 20180909-03 was isolated from a sesame spot leaf sample in Henan province, China, in 2018. dsRNA was extracted from 0.2g fungal mycelia by using CF-11 cellulose column chromatography [20]. To remove DNA and ssRNA, we treated the dsRNA sample with RNase free DNaseI and S1 nuclease (TaKaRa Dalian, China). The dsRNA sample was analyzed by 1.2% (w/v) agarose gel electrophoresis. After treating the crudely extracted dsRNA with DNaseI and S1 nuclease, a single band of approximately 5 kb was observed (supplementary Figure S1).

To further analyze dsRNA virus, we performed Next Generation Sequencing of the *C. cassiicola* strain. Total RNA of the mycelium was extracted using RNAiso Plus (TaKaRa Dalian, China). Paired-end sequencing libraries were prepared and sequenced on an Illumina HiSeq 2500 platform at Shanghai Bohao Biotechnology. In order to obtain clean sequences, the original reads of deep sequencing were processed by removing adaptor sequences and low-quality reads. Then, the clean reads were assembled through de novo in CLC Genomics Workbench (version 6.0.4) to form contigs. Those contigs, which have a high degree of matching with the virus of the NCBI database by using BLASTx (https://www.ncbi.nlm.nih.gov/), are identified as potential virus sequences. From these contigs, we found that the length of contig1246 which belongs to the *Victorivirus* genus was about 5kb.

According to the user manual of PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa, China), the cDNA of strain 20180909-03 was synthesized. According to the putative viral sequence, the full length of the contig1246 was verified through the assembling of 11 pairs of specific primer amplified fragments (supplementary Table S1). To complete the 5'- and 3'-terminal genomic sequences, rapid amplification of cDNA ends was performed by using a SMARTer RACE 5'/3' Kit (TaKaRa, China). GSP-R1 (5'-ACCCGCCACCGCATTAGCCAGGA -3') was used for the 5'-RACE reaction. GSP-F1:(5'-GCGTCCTCGCCGATTACCCCGTG-3') and GPS-F2:(5'-CGAAGACCAAGAGCGACAATC-3') were used by nested PCR for the 3'-RACE reaction. All PCR products were purified by FastPure Gel DNA Extraction Kit (Vazyme, China) and cloned into the pMD™18-T vector and introduced into JM109 Chemically Competent Cell (TSINGKE, China) by transformation. At least three recombinant clones were selected for sending to Sangon Biotech for sequencing to verify the nucleotide sequence accuracy.

Whole genome sequence of the CcVV1 analysis and alignment were performed using ORF Finder and CD-search in the NCBI (https://www.ncbi.nlm.nih.gov/), DNAMAN and MEGA (7.0 version). The phylogenetic trees were constructed by the maximum-likelihood (ML) method with 1000 bootstrap replicates [21]. The GC content was determined by NoVopro (https://www.novopro.cn/tools/gc-content.html). The Expasy was used to calculate the protein molecular weight (Mw) and isoelectric point (pI) (https://web.expasy.org/compute_pi/). The RNA Folding Form was used to find potential secondary structures in the terminal sequences of the CcVV1 (http://www.unafold.org/mfold/applications/rna-

folding-form.php). The Gene structure was drawn through the Illustrator for Biological Sequence(http://ibs.biocuckoo.org/dbvisualization.php). The H-type RNA pseudoknot was predicted by using an available website (https://dotknot.csse.uwa.edu.au/).

Sequence Properties

Sequence analysis revealed that *C. cassiicola* strain 20180909-03 contained a dsRNA virus belonging to the *Victorivirus* genus. This dsRNA virus was discovered for the first time in *C. cassiicola*, so it was named Corynespora cassiicola Victorivirus 1 (CcVV1).

The complete sequence of CcVV1 (GenBank accession number: OK317696) is 5140nt and it has a GC content of 56.87% (Figure 1a). Sequence analysis shows that CcVV1 has two large open reading frames (ORF), which encode different proteins: ORF1 (303-2570nt) encodes CP, and ORF2 (2567-5050nt) encodes RdRp. Besides, the 5' terminus of the whole genome contains two small ORFs (95-184nt and 197-301nt), but they have no sequence similarity with the NCBI database. The start codon of ORF2 overlaps with the stop codon of ORF1 in the tetranucleotide AUGA (2567-2570nt). In addition, a H-type pseudoknot structure was found in the upstream part of the AUGA motif, which was believed to be related to the translation of the downstream ORF2 (Figure 1b). And the untranslated regions (UTRs) at the 5' and 3' ends are 94nt and 90nt long respectively and are predicted to have stable secondary structures (Figure 1c 1d).

The CP encoded by ORF1 contains 755 amino acids, and it was predicted that the molecular weight of the protein was 79.37 kDa and the isoelectric point was 6.25. The CP of ORF1 has the highest sequence similarity to the putative CP encoded by Beauveria bassiana victorivirus NZL/1980 (BbVV_NZL/1980; YP_009032632.1, 74% identity, E-value 0). We also found an Ala/Gly/Pro-rich region in the C-terminal sequence of CcVV1 (Figure 2a), which is a significant molecular feature of the *Victorivirus* genus[15].

A phylogenetic tree based on the CP sequences of CcVV1 and 15 other dsRNA viruses of *Totiviridae* was constructed by the ML method (Figure 2b). The CP of CcVV1 formed a branch with the CP members of six viruses representing the *Victorivirus* genus, which showed that the CP of CcVV1 had closer relationships with members of the *Victorivirus* genus.

The RdRp encoded by ORF2 contains 827 amino acids and the molecular weight of the protein and pI were predicted to be 90.47 kDa and 8.76 respectively. The RdRp has the highest sequence similarity to the putative RdRp encoded by Sclerotinia nivalis victorivirus 1 (SnVV1;YP_009259368.1, 74% identity, E-value 0). Multiple sequence analysis showed that the RdRp sequence of CcVV1 contained eight conserved motif [13] (Figure 3a). A phylogenetic analysis based on the amino acid sequence of putative RdRp encoded by CcVV1 also showed close relationships with members of the genus *Victorivirus* (Figure 3b).

According to our phylogenetic and gene structure analysis, the mycovirus CcVV1 showed close relationship with the other reported victoriviruses[22]. Thus, all these results suggest that CcVV1 is a new

member of the genus *Victorvirus*. To the best of our knowledge, it is also the first reported victorivirus infecting the fungus *C. cassiicola.*

Declarations

Compliance with ethical standards

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Conflict of interest All authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

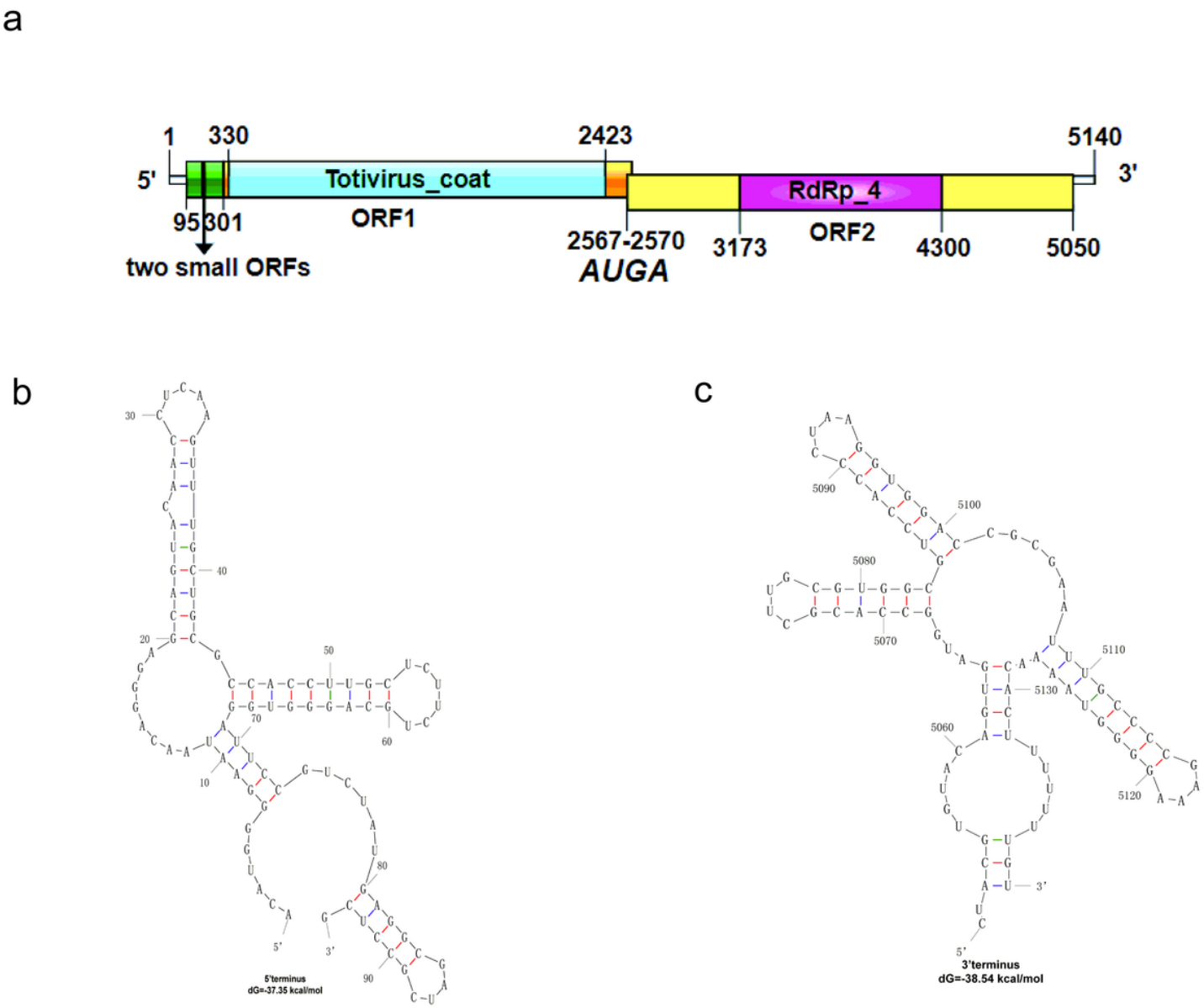

Figure 1

(a) Genome organization of Corynespora cassiicola Victorivirus 1 (CcVV1). (b) The H-type RNA pseudoknot predicted upstream of the tetranucleotide sequence AUGA of CcVV1. (c) Schematic representation of the predicted secondary structures for the 5’-UTR. (d) Schematic diagram of the predicted secondary structures for the 3’-UTR

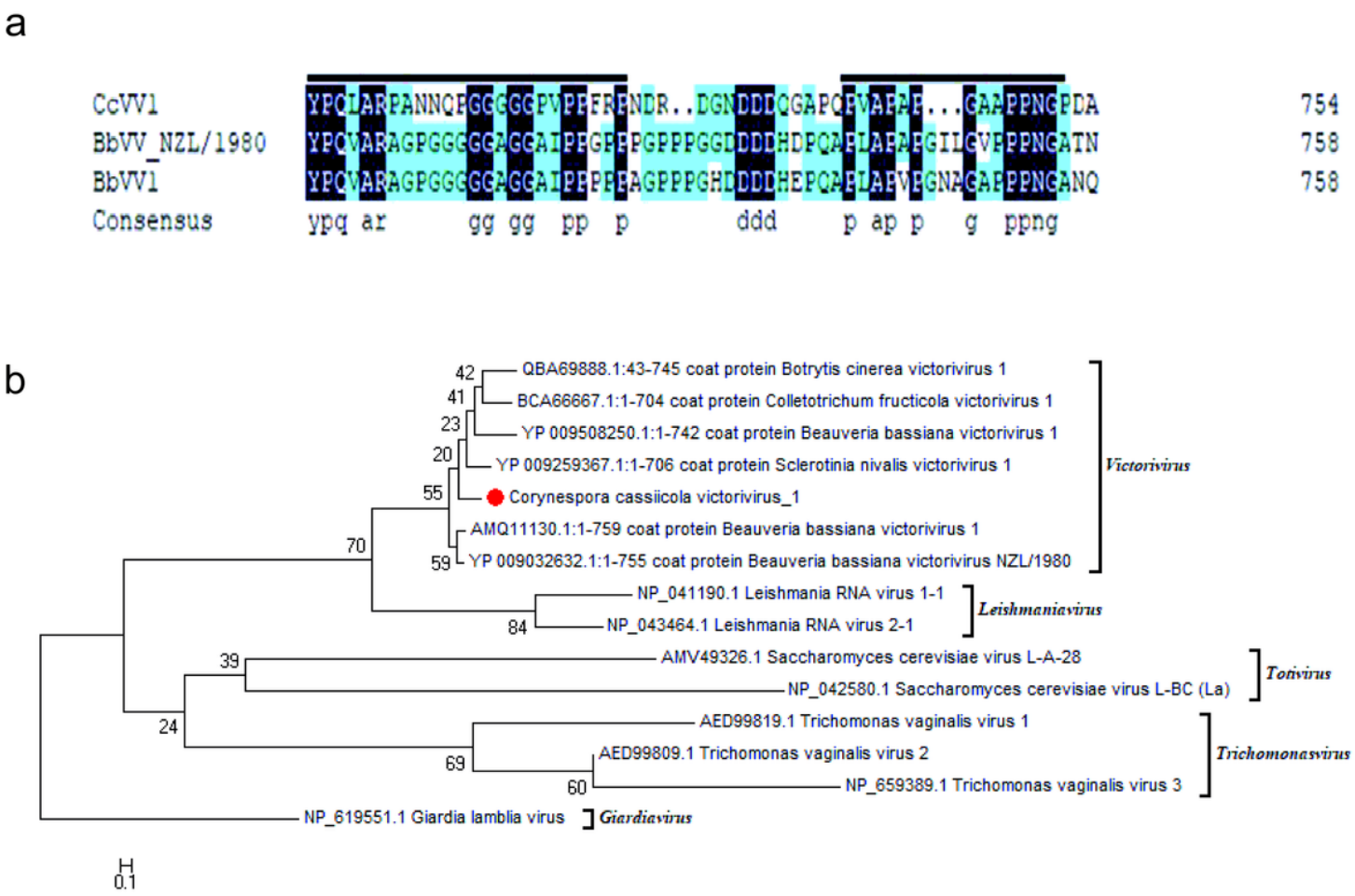

Figure 2

(a) Multiple alignments of the amino acid sequences of the C-terminal sequence of CP and other homologous victorivirus. Abbreviations are as follows: Beauveria bassiana victorivirus NZL/1980 (BbVV_NZL/1980; YP_009032632.1); Beauveria bassiana victorivirus 1 (BbVV1; AMQ11130.1). (b) Phylogenetic tree of viruses of the family Totiviridae based on CP amino acid sequences

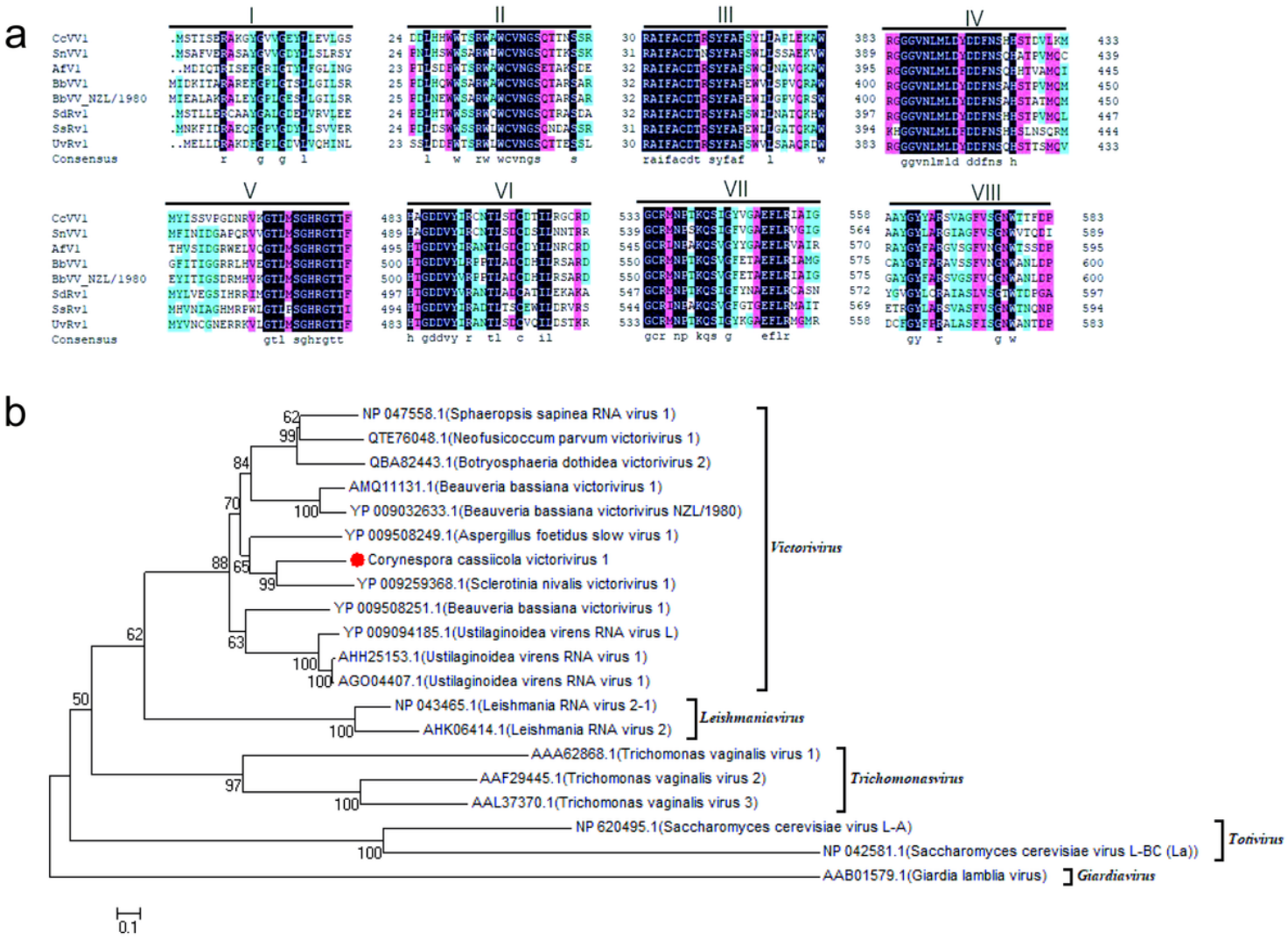

Figure 3

(a) Multiple alignment of the amino acid sequence of the RdRp encoded by CcVV1 and other related victorviruses. Conserved motifs are indicated as I–VIII. Abbreviations are as follows: Sclerotinia nivalis victorivirus 1 (SnVV1; YP_009259368.1); Aspergillus foetidus slow virus 1 (AfV1; YP_009508249.1); Beauveria bassiana victorivirus 1 (BbVV1; AMQ11131.1); Beauveria bassiana victorivirus NZL/1980 (BbVV_NZL/1980; YP_009032633.1); Soybean-associated double-stranded RNA virus 1 (SdRv1; ALM62239.1); Sphaeropsis sapinea RNA virus 1 (SsRv1; NP_047558.1); Ustilaginoidea virens RNA virus 1 (UvRv1; AHH25153.1). (b) Phylogenetic tree of viruses of the family Totiviridae based on RdRp amino acid sequences.

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