Molecular Characterization of a Novel Partitivirus and a Fusarivirus Co-infected in the *Nigrospora Sphaerica* Fungus

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Abstract

Here we reported the molecular characterization of two novel mycoviruses co-infected in a plant pathogenic fungus, *Nigrospora sphaerica* that were designated as Nigrospora sphaerica fusarivirus 1 (NsFV1) and Nigrospora sphaerica partitivirus 1 (NsPV1), respectively. NsFV1 has an undivided genome of 6,147 bp, excluding the polyA tail, and was predicted to contain two nonoverlapping open reading frames (ORF1 and 2). The larger ORF1 encoded a polyprotein containing a conserved RNA-dependent RNA polymerase (RdRp) and a helicase domain that have functions for RNA replication, and the smaller ORF2 encoded a putative protein with an unknown function. The NsPV1 was consists of two genome segments, which were in lengths of 1,796 bp and 1,455 bp, respectively. Each of the two dsRNAs had a single ORF and were deduced to encode proteins with homology to viral RdRp and coat protein (CP), respectively, in the family *Partitiviridae*. Phylogenetic analysis showed that NsFV1 was placed within the newly proposed family Fusariviridae, while NsPV1 was belonging to the genus *Gammapartitivirus* in the family *Partitiviridae.* This was the first description of mycovirses infected the fungus *N. sphaerica.*

Introduction

Mycoviruses are viruses residing and infecting almost all major taxa of fungal groups, including filamentous fungi, yeasts, and oomycetes [1–4]. Since the first report of mycovirus infecting the cultivated button mushroom *Agaricus bisporus* as a pathogen, a number of mycoviruses have been identified, especially via application of high-throughput sequencing technique [5]. Most mycoviruses were confirmed to infect their host latently, but some exerted clearly phenotypic alterations in their host, such as induced hypovirulence. These hypovirulence associated mycoviruses were potential biological agents for control of disease caused by pathogen fungi, as has been exampled by the successfully use of Cryphonectria hypovirus 1 (CHV1) to control chest blight disease in Europe [2, 3, 6]. In addition, some mycoviruses also have beneficial effects on their host fungus [7, 8]. For example, a virus named Curvularia thermal tolerance virus which was identified in an endophytic fungus could enhance heat tolerance to the host plant [9]. Recently, some reports suggested the positive interactions between mycoviruses and their fungal hosts. For example, two distinct partitiviruses have been reported to enhance the osmotic tolerance of *C. parasitica* and mycotoxin production of *Aspergillus ochraceus*, respectively [10, 11]. In addition, a partitivirus infecting an isolate of *Trichoderma harzianum* could enhance the antagonistic activity of its host [12]. Mycoviruses have aroused wide interests among researchers for biological control against pathogenic fungi and for biotechnological applications, such as in elucidating the pathogenic mechanisms of pathogenic fungi [3, 6]. Thus, many mycoviruses have been identified during the last few decades, which also in turn expanded our knowledge on mycoviral diversity, evolution and ecology [3, 13].

Mycoviruses have their genomes of double-stranded (ds) RNA [3], circular single-stranded (ss) DNA [14, 15], negative-sense single-stranded (-ss) RNA [16], positive-sense single-stranded (+ ss) RNA linear genomes and reverse-transcribing RNA linear genomes [3]. Viruses in the family *Partitiviridae* comprised of two dsRNA segments, in sizes ranging from 1,300 bp to 2,500 bp, putatively encoding proteins of RNA-

dependent RNA polymerase (RdRp) and coat protein (CP), respectively. Partitiviruses form isometric, non-enveloped particles with 30–35 nm in diameter. Currently, the *Partitiviridae* family was divided into five genera: *Alphapartitivirus*, *Betapartitivirus*, *Gammapartitivirus*, *Deltapartitivirus* and *Cryspovirus* [17].

Fusariviridae is a + ssRNA virus family proposed recently to be created with the FgV1 as the prototype [18, 19]. Viruses in this family typically have genomes in size ranging from 6 to 10 kb and contain one large open reading frame (ORF) and one to three smaller ORFs. The larger ORF encoded a replicase-related protein containing RdRp and helicase (Hel) domains, while the other smaller ORFs encoded proteins with unknown functions [18, 20].

Nigrospora sphaerica is a kind of saprophytic, endophytic and phytopathogenic fungus that distributed widely in nature, even in insects [21, 22]. As a plant pathogen, this fungus can infect an amount of plants causing leaf spot, leaf blight or shot hole diseases [23]. As endophytic fungus, some *N. sphaerica* strains could produce a variety of secondary metabolites with antimicrobial ability [24]. Although a few mycoviruses have been reported in the *Nigrospora* species [25–28], however, to date, no virus has been reported in the *N. sphaerica.*

In this study, we reported two novel mycoviruses from a *N. sphaerica* strain Ns2-3 that were designed as Nigrospora sphaerica fusarivirus 1 (NsFV1) and Nigrospora sphaerica partitivirus 1 (NsPV1), respectively. Genomic organization and phylogenetic analyses indicated that NsPV1 and NsFV1 were novel fusarivirus and partitivirus, respectively.

Provenance Of The Virus Material

The *N. sphaerica* strain Ns2-3 was collected from diseased pepper plant and its parasite *bemisia tabaci* from Hunan Province of China, in 2018. It was cultured on potato dextrose agar plates at 26°C. The mycelial mass was harvested after 7 days of culture in PD broth with shaking (170 rpm) at 26°C. DsRNA was extracted using the CF cellulose chromatography method as described by Morris and Dodds [29]. The dsRNA extractions were treated with S1 nuclease and DNase I for elimination of any contaminated DNA and ssRNA molecules, and then separated in a 1% (w/v) agarose gel. DsRNA fragments were individually purified and applied to cDNA cloning. The cDNA libraries were constructed using reverse transcriptase and random hexanucleotide primers. Sequences were completed by filling the gaps using RT-PCR amplification and by obtaining the dsRNA terminals using adaptor ligation-mediated PCR amplification [30]. All the PCR products were ligated into the pMD18-T vector (TaKaRa, Dalian, China), cloned and sequenced. Every base of the sequences was determined in at least three independent clones. The resulting sequences were finally assembled and subjected for sequence analysis.

Potential open reading frames (ORFs) and conserved domain(s) were found on the National Center for Biotechnology Information (NCBI) database using the ORF finder and conserved domain search programs, respectively. Homology searches were performed using the BLASTp program. Multiple sequence alignment and phylogenetic analysis were conducted by the CLUSTALX [31] and MEGA 6 programs [32], respectively.

Sequence Properties

From the assembled virus clones, the full length cDNA sequences of three dsRNA segments were obtained. Subsequent sequence analysis indicated that the 6-kbp large segment was the genome of a novel fusarivirus that we named NsFV1 (Accession number: MT774523), while the other two dsRNA segments, in approximately 2-kbp, constituted the genome of a novel partitivirus, NsPV1 (Accession number: MT774524 and MT774525). Schematic diagram of genomic organization of the two viruses were represented in Fig. 1A and Fig. 2A,respectively.

The complete genome of NsFV1 was 6,147 bp (excluding the polyA tail), with a G + C content of 52.4%. It contained two ORFs (ORF1 and 2) on the genomic RNA, preceded and followed by untranslated regions (UTRs) of 77 nt and 53 nt, respectively. NsFV1 ORF1 encoded a 1,508 amino-acid (aa) polyprotein with a calculated molecular mass of 169.2 kDa. Conserved domain search against the NCBI database revealed the presence of an conserved RdRp (RdRP_1, pfam00680) and a helicase (DEAD, pfam00270) domains in the ORF1-encoded polyprotein. Eight conserved motifs were found in the conserved RdRp domain by multiple aa sequence alignment and comparison between the viral RdRps between NsFV1 and other selected viruses (Fig. 1B). Homology search, using the BLASTp, indicated that the ORF1-encoded protein of NsFV1 had significant sequence identities to the proteins encoded by other putative fusariviruses, such as Gaeumannomyces tritici fusarivirus 1 (GtFV1), Plasmopara viticola associated fusarivirus 2 (PvAFV2), Penicillium aurantiogriseum fusarivirus 1 (PaFV1). The RdRp domain of NsFV1 showed 38.87–63.83% aa sequence identities to the corresponding domains of other fusariviruses, with GtFV1 being the best math displaying 63.83% aa identity (Query coverage: 99%; E value: 1e-142). In addition, the RdRp domain of NsFV1 also showed moderate levels of sequence identity (Identity: 25.55%-28.43%; Query coverage: 64%-92%) to that of the hypoviruses in the family *Hypoviridae*, and that of other related viruses. Similarly, the Hel domain of NsFV1 had a significant degree of aa sequence identity to those of the fusariviruses, ranging from 29.77–58.09% (Query coverage: 73 to 100%). However, the NsFV1 Hel domain had no sequence identity to those of the viruses in the family *Hypoviridae*, but had low sequence identity to some bacterial RNA helicases. NsFV1 ORF2 was predicted to encode a 45.8 kDa protein with 411 aa. Database search revealed that the ORF2-encoded protein was homologous only to the hypothetical protein encoded by GtFV1, and showed no significant similarity to any other known viral proteins. However, a putative conserved domain of SMC (structural maintenance of chromosomes, COG1196) was found in the N-half of the ORF2 encoded protein, which was predicted to be associated with cell cycle control, cell division and chromosome partitioning. The SMC proteins were found in bacteria, archaea and eukaryotes. However, some viruses including fusariviruses also contained the SMC domain as described previously [33]. Its occurrence in mycovirus is noteworthy and might reveal the horizontal gene transfer between viruses and other eukaryotes or prokaryotes. In the other hand, the presence of SMC in different viruses might also indicate the common origin of these viruses. The true function and evolution of the SMC domain in the NsFV1 ORF2 needed further study.

In order to estimate the phylogenetic relationships between NsFV1 and other mycoviruses, we conducted phylogenetic analysis based on aa alignments of the RdRp and Hel domains. The phylogenetic tree

generated based on the RdRp domain alignment showed that NsFV1 was clustered in a fusarivirus-clade, including the prototype FgV1, and distinct from members of the family *Hypoviridae* (Fig. 1C). A phylogenetic tree of the Hel domain also showed that NsFV1 was grouped in a clade and exhibited a similar tree topology with the RdRp based phylogenetic analysis (Fig. S1). Overall, based on the similarities in conserved RdRp and Hel domains and phylogenetic analysis, we can suggest the NsFV1 as a novel member of the proposed family Fusariviridae.

Sequences analysis indicated that the NsPV1 was composed of two dsRNA segments, which were designated as dsRNA 1 and dsRNA 2, respectively. NsPV1 dsRNA 1 was 1,796 bp in length, with a G + C content of 47.6%, while the dsRNA 2 was 1,455 bp long, containing a 51.2% G + C content. The 5' and 3' untranslated regions (UTRs) were 104 bp and 75 bp in dsRNA 1, 92 bp and 100 bp in dsRNA 2, respectively. The 5’ and 3’UTRs between the dsRNA 1 and dsRNA 2 were conserved, possessing an identical stretch of 5’-CGTGAAATAC-3’ in the 5’-terminal and a stretch of 5’-TAAACCAAAA-3’ in the 3’-terminal (Fig. 2B), which were considered to be important for replication of viruses with multicomponent RNA genome [34].

The coding strand of the NsPV1 dsRNA 1 contained a single ORF encoding a 538 aa protein with an estimated molecular mass of 62.0 kDa. Homology search revealed that the dsRNA 1 encoded protein shared sequence similarity with RdRp proteins encoded by other partitiviruses. Magnaporthe oryzae partitivirus 1 (MoPV1) was the most closely related virus, showing aa sequence identity of 70.50% (E value: 0; query cover: 100%), followed by Magnaporthe oryzae partitivirus 2 (MoPV2, identity: 68.65%; E value: 0; query cover: 100%), Penicillium stoloniferum virus F (PsV-F, (identity: 67.23%; E value: 0; query cover: 99%) and Pythium nunn virus 1 (PnV1, identity: 55.51%; E value: 0; query cover: 95%). In addition, conserved motifs characteristic of RdRp sequences of other members of the *Partitiviridae* were found in the NsPV1 encoded RdRp. NsPV1 dsRNA 2 contained a single ORF, which was predicted to encode a putative 46.5 kDa protein comprised of 420 aa. The 46.5 kDa protein shared the maximal aa identity of 65.53% (E value: 0; query cover: 96%) to that of MoPV1, followed by MoPV2, PsV-F and PnV1, with the aa identities ranging from 37.50–65.28%. Interesting, the 46.5 kDa protein also showed a lower level of aa sequence identity to proteins of other eukaryote, including *Gracilariopsis chorda*, *Oidium neolycopersici* and *Erysiphe necator*. A phylogenetic tree was constructed using the RdRp of NsPV1 and other partitiviruses (Fig. 2C). It revealed that NsPV1 was placed in a branch including members of the genus *Gammapartitivirus*, as expected from the homology search result.

The species demarcation criteria of partitiviruses are that aa identities in the RdRp and CP is fewer than 90% and 80%, respectively [16]. Since the aa identities of RdRp and CP between NsPV1 and the closely related MoPV1 were lower than the cutoff values, we can suggest the classification of NsPV1 as a novel species of the genus *Gammapartitivirus* in the family *Partitiviridae.*

In conclusion, we identified two novel mycoviruses from a *N. sphaerica* strain Ns2-3. Sequence similarity, genome organization and phylogenetic analysis supported the affiliation of NsFV1 and NsPV1 as novel members of the family Fusariviridae and *Partitiviridae*, respectively. As far as we know, this was the first

report of mycoviruses co-infected in the fungus *N. sphaerica.* The biological effects of these viruses on the fungal host and the functions of the virus genes in terms of virus-host interactions remained further study.

Declarations

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

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

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Figures

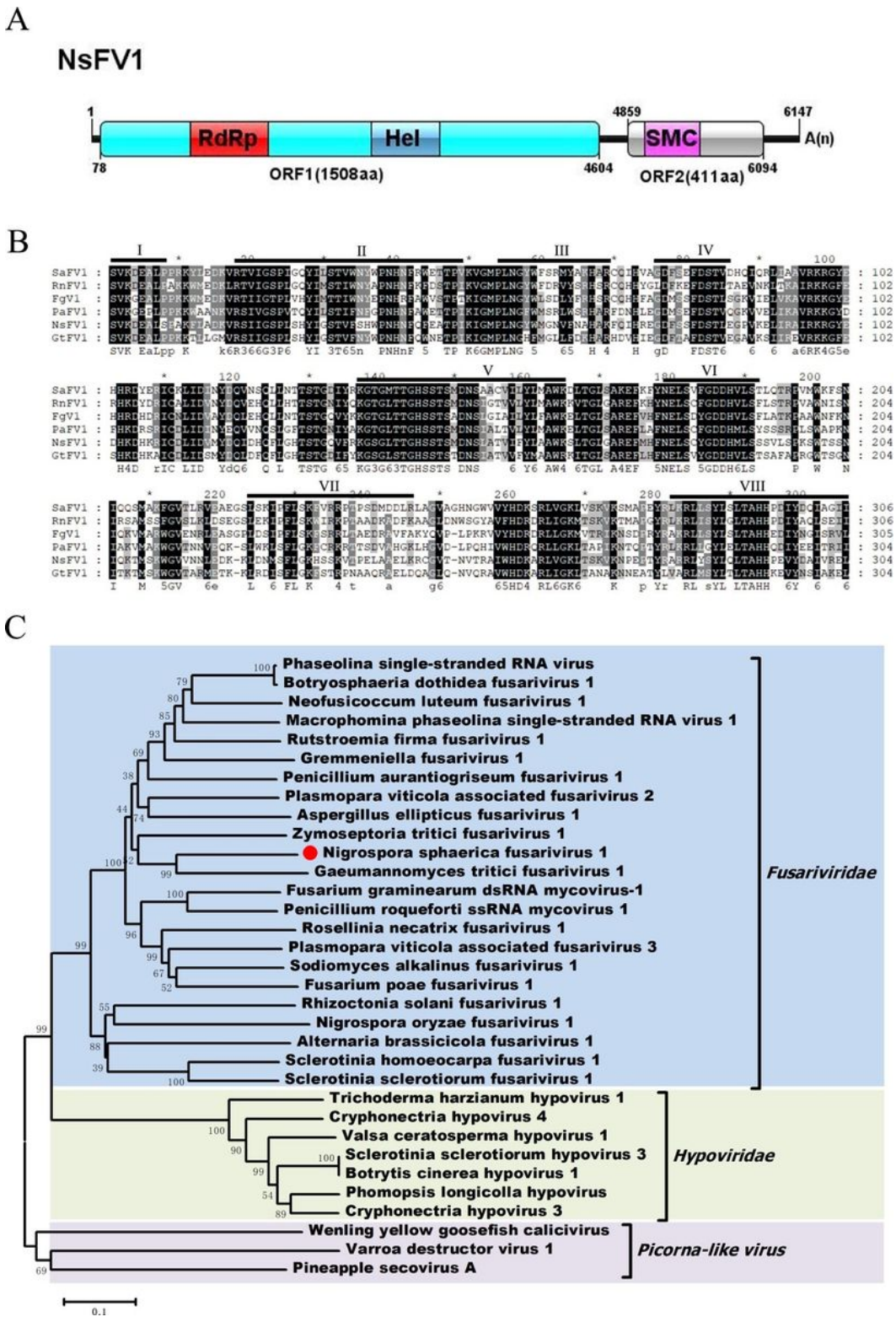

Figure 1

(A). Genomic organization of NsFV1. The open reading frames (ORF1 and 2) and untranslated regions (UTRs) were indicated by colored boxes and solid lines, respectively. ORF1 contained the conserved domains of RNA-dependent RNA polymerase (RdRp) and RNA helicase (Hel), and the ORF2 contained the SMC conserved domain. (B) Multiple alignments of amino-acid (aa) sequences of the RdRp domains of NsFV1, SaFV1, RnFV1, FgV1, PaFV1 and GtFV1 that contained the conserved motifs characteristic of

positive-sense single-stranded (+ss) RNA viruses. The alignment was carried out using the CLUSTALX program and showed using the GeneDoc program. Conserved motifs in the RdRps are delegated by bold black lines and the Roman numerals I–VIII. (C) Phylogenetic analysis of virus NsFV1. The neighbor-joining phylogenetic tree was constructed based on the RdRp sequences using the MEGA6. Bootstrap values were determined with 1000 re-samplings. The GenBank accession numbers of the viral proteins used for phylogenetic tree construction were shown in Table S1.

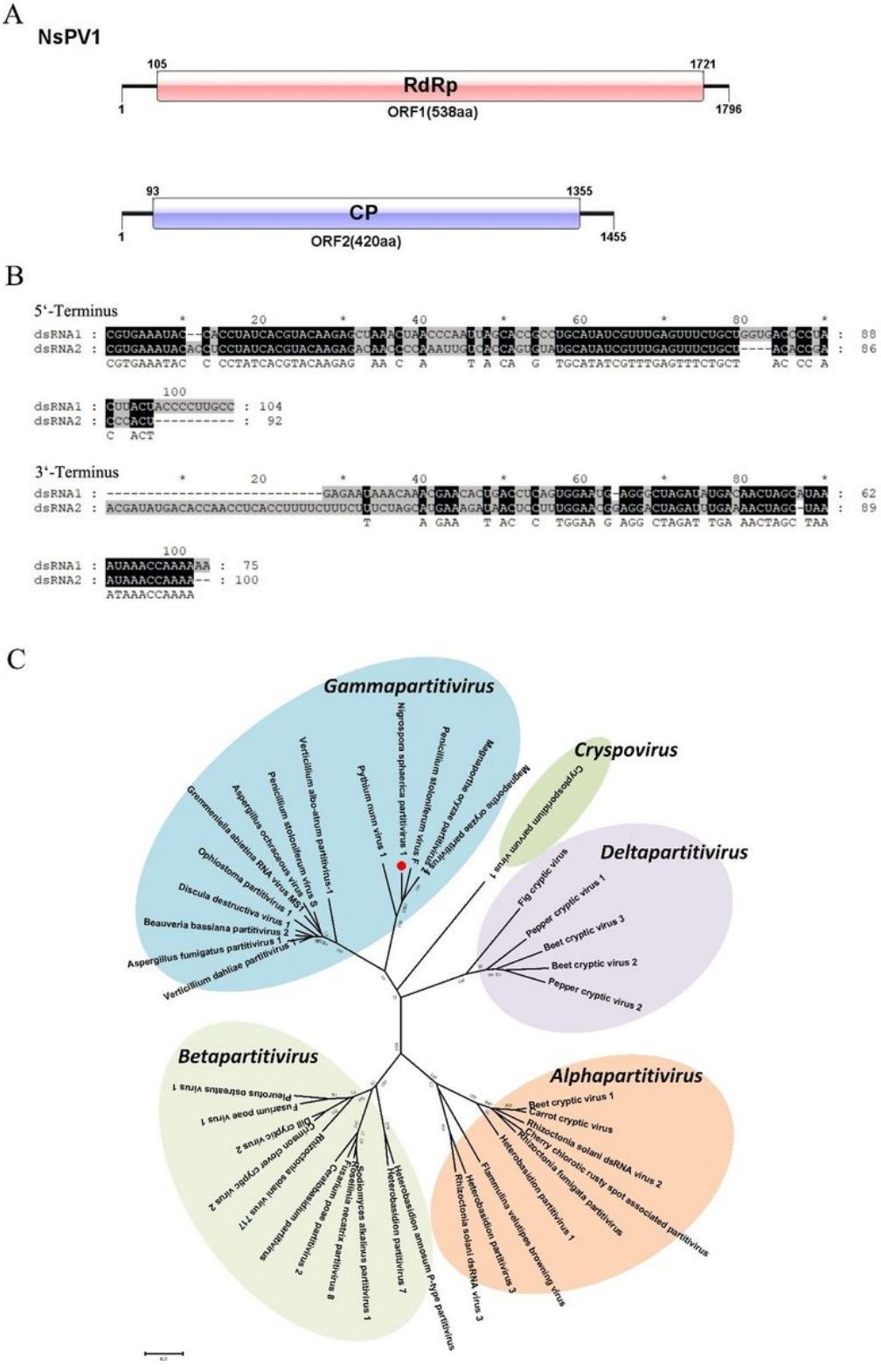

Figure 2

(A) Schematic representation of genome of NsPV1. The RdRp and CP were also displayed as colored boxes. The 5’ and 3’ UTRs were indicated as solid black lines. (B) Alignments of the nucleotide sequences of 5’ and 3 UTRs between the two dsRNA segments of NsPV1. Black shading indicated the identical nucleotides between the dsRNA 1 and dsRNA 2. (C) Phylogenetic analysis of NsPV1. The phylogenetic tree was generated by neighbor-joining method using the RdRp aa sequences of NsPV1 and other selected members of the family Partitiviridae. NsPV1 was indicated in the phylogenetic tree. The scale bars indicated a genetic distance of 0.2 amino acid substitutions per site. Virus names and their corresponding GenBank accession numbers were shown in Table S2.

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