A second capsidless hadakavirus strain with a 10 positive-sense single-stranded RNA genomic segments from *Fusarium nygamai*

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Abstract

A unique capsidless virus with a positive-sense, single-stranded RNA genome (hadakavirus 1, HadV1), a member of the extended picorna-like supergroup, has previously been isolated from the phytopathogenic fungus, *Fusarium oxysporum*. This study describes the molecular and biological characterisation of a second hadakavirus strain from *Fusarium nygamai*, which has not been previously explored as a virus host in detail. The virus termed hadaka virus 1 strain 1NL (HadV1-1NL) has features similar to the first hadakavirus, HadV1-7n, despite the difference in segment number 10 for HadV1-1NL vs. 11 for HadV1-7n. The ten genomic RNA segments of HadV1-1NL range in size from 0.9 kb to 2.5 kb. All HadV1-1NL segments show 67% to 86% nucleotide sequence identity to the HadV1-7n counterparts, whereas HadV1-1NL has no homolog to HadV1-7n RNA8 encoding a zinc-finger motif. Another interesting feature is the possible coding-incapability of HadV1-1NL RNA10. The capsidless nature of HadV1-1NL replicative form dsRNA was predicted based on its RNase A susceptibility. Phenotypic comparison of multiple virus-infected and virus-free single spore isolates indicates asymptomatic infection by HadV1-1NL. Less efficient vertical transmission via spores was observed as infected fungal colonies, from which spores were derived, became older, as was observed for HadV1-7n. This study shows a second example of the hadakavirus which probably has a peculiar virus lifestyle.

Introduction

The megataxonomy of RNA viruses has been established in which five groups accommodate all viruses with RNA-directed RNA polymerase (RdRP) genes as the hallmark [19, 50]. The five groups, mostly monophyletic, are now officially classified as phyla *Lenarviricota* (also known as branch 1), *Pisuviricota* (branch 2), *Kitrinoviricota* (branch 3), *Duplornaviricota* (branch 4), and *Negarnaviricota* (branch 5) by the International Committee on Taxonomy of Viruses [46]. The phylum *Pisuviricota* (branch 2), also known as the extended picornavirus supergroup, attracts great attention because this group, unlike the other four groups, include both single-stranded (ss), positive-sense (+) RNA viruses and double-stranded (ds) RNA viruses [17, 18]. Furthermore, branch 2 accommodates a number of fungal viruses (mycoviruses) such as hypoviruses, partitiviruses, amalgaviruses and fusariviruses with (+)RNA or dsRNA genomes, in addition to human, animal and plant viruses. Among them are peculiar mycoviruses with intermediate properties between (+)RNA viruses and dsRNA viruses exemplified by polymycoviruses [15, 20, 32] or with unique lifestyles such as yadokariviruses [12, 53] and Hadaka virus 1 (HadV1) [33].

Polymycoviruses and HadV1 are phylogenetically closely related based on RdRP alignment. Polymycoviruses have four- to eight-segmented RNA genomes generally with conserved terminal sequences and encode proline-alanine-serine rich proteins (PASrps) that can bind nucleic acids in a sequence non-specific manner when tested *in vitro* [32]. All polymycoviruses reported thus far encode PASrps [14, 15, 20, 52] and three other conserved genes encoding RNA-dependent RNA polymerase (RdRP), an unknown protein (containing transmembrane and zinc finger motif), and methyltransferase (MTR). Polymycovirus PASrps are associated with their genomic dsRNAs and are believed to form an RNA-protein complex (PASrp-associated form) [15] or filamentous particles [14]. Surprisingly, these complexes with dsRNA and even purified deproteinised dsRNA were shown to be infectious when experimentally introduced into protoplasts of their host fungi, characteristics classifying polymycoviruses into dsRNA viruses. These properties of polymycoviruses contrast those of HadV1. The HadV1 genome possesses 11 RNA segments with conserved terminal sequences and one of the segments encodes RdRP with greater affinity to (+)RNA viruses than to dsRNA viruses. Importantly, HadV1 does not encode PASrp and appears to exist in a fully capsidless form. HadV1 dsRNA segments (a replicative form) in mycelial homogenates in neutral buffer are accessible by RNase under the conditions in which no polymycovirus dsRNA is digested. In addition, no HadV1 complex was pelleted by ultracentrifugation, whereas the polymycovirus dsRNA-protein complex could be pelleted in the same conditions. These prompted Sato and others to propose that HadV1 is a capsidless (+)RNA virus with 11 genomic segments [33].

Our group started screening collections of Pakistani fungal isolates that were previously collected from plants and soils for viruses before [13, 32, 34]. During the course of the study, HadV1 was discovered

from a Pakistani isolate of the phytopathogenic ascomycete *Fusarium oxysporum* (family Nectriaceae, order Hypocreales). *Fusarium* spp. are cosmopolitan and contain destructive phytopathogenic fungi which cause major economic losses and are represented by *F. graminearum* and *F. oxysporum* [7]. This fungal genus is a complex of different species which are difficult to distinguish morphologically [1]. Herein we report on the second strain of the proposed species Hadakavirus nanga, with a deca-segmented (+)RNA genome, hadaka virus 1-1NL (HadV1-1NL), from *Fusarium nyagamai* (isolate 1NL). HadV1-1NL lacked a genomic segment homologous to HadV1-7n RNA8. Shared similarities include the conserved terminal sequences, with an amino acid sequence identity ranging from 49.4% to 92.3% between corresponding proteins, and higher vertical transmission rates through spores borne on relatively young colonies than through those on old colonies. This is the second report of mycovirus which showed a resemblance to polymycoviruses but lacks the proline-alanine-serine-rich protein (PASrp) and possibly has a peculiar virus lifestyle.

Materials and Methods

Fungal isolates and culture conditions

The fungal isolate termed 1NL was collected from diseased leaves of a household pomegranate plant in District Mianwali, Province Punjab, Pakistan. The screening was carried out during 2018. The diseased leaves were surface-sterilized with 1% sodium hypochlorite followed by a three-time wash with sterile distilled water as previously described [10]. The infected portion of a leaf was cut, placed on Difco potato dextrose agar (PDA, Becton, Dickinson and Co.) media, and cultured at room temperature (25ºC). Fungus isolated from the leaf piece was regularly sub-cultured on PDA plates at room temperature or stored at -80ºC in 10% glycerol. The fungal isolate was identified as *Fusarium nygamai* based on sequencing of the internal transcribed spacer (ITS) region [48], the intergenic spacer (IGS) region [2], and a translation elongation factor 1 gene (*ef1α*) [30] followed by a BLASTN search of the "Nucleotide collection (nr/nt)" database and phylogenetic analyses. Primer sequences are listed in Supplementary Table S1. IGS and *ef1α* sequences used for the phylogenetic analyses are listed in Supplementary Tables S2 and S3. The isolate 1NL is infected by hadaka virus 1 strain 1NL (HadV1-1NL) (see Results).

A virus-free fungal strain, 1NL-VF1, isogenic to *F. nygamai* isolate 1NL was obtained from conidial sub-isolates of the 1NL (see the section "***Vertical transmission and colony morphology***" below). *Fusarium oxysporum* isolate 7n that was infected with the first hadakavirus strain (HadV1-7n) [33] was used to compare HadV1-1NL with HadV1-7n. These fungal isolates as well as 1NL, were cultured under the same conditions.

dsRNA extraction and electrophoresis

Fungal isolates were cultured on a PDA-cellophane plate for three days. The dsRNA-enriched fraction was prepared from the culture by the cellulose column chromatography method [9]. The dsRNA-fraction was further treated with RQ1 DNase (Promega Corp.) and S1 Nuclease (Thermo Fisher Scientific Inc.). The purified dsRNA was then subjected to electrophoresis in agarose (1%) or SDS-polyacrylamide (10%) gels. Agarose gel electrophoresis was performed in 0.5× TBE. The SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as previously described [36]. dsRNA bands on the gels were stained with 0.1 mg/mL ethidium bromide solution and visualised with a UV transilluminator.

Nucleotide sequencing of viral RNA

For the viral genome sequencing, Next Generation Sequencing (NGS) was performed by Macrogen Inc (Tokyo, Japan). Total and dsRNA extractions were performed from the fresh cultures on cellophane, using cellulose (Advantech, Tokyo, Japan) [9]. Total RNA and dsRNA preparations from three fungal isolates (which includes *F. nygamai*, *Geotrichum* sp. and *Neofusicoccum parvum*) were pooled together (total 85.3 µg, RNA integrity number =8.1) and sent for analysis. After the rRNA depletion, RNA-seq with the Illumina platform (HiSeq 2500, 100 bp pair-end reads), a total of 40,474,312 reads in total were assembled *de novo* into 43,876 contigs by using CLC Genomics Workbench version 11 (CLC Bio-Qiagen, Aarhus, Denmark). Sequence mining was performed by using local BLAST with the reference-viral sequences from National Center for Biotechnology Information (NCBI) or specific viral sequence as queries. Virus sequence-specific primers designed from the contigs obtained from RNA-seq were used for the detection of viruses in each fungal isolate. Viral sequences from the other two fungal isolates (*Geotrichum* sp. and *N. parvum*) will be reported elsewhere (Khan et al., unpublished data). Nucleotide sequences of the 5′- and 3′-terminals of the dsRNA were determined by RNA ligase-mediated rapid amplification of cDNA ends (3′ RLM-RACE) as described previously [40]. Both 3′ terminals of dsRNA were ligated with a 3′-RACE adaptor (5′ phosphorylated oligodeoxynucleotide, 5′-PO_4-CAATACCTTCTGACCATGCAGTGACAGTCAGCATG-3′) at 16–18 ºC. The ligated RNA was used for reverse transcription with the 3′-RACE-1st primer (5′-CATGCTGACTGTCACTGCAT-3′). The synthesised cDNA was used as a template for PCR with and 3′-RACE-2nd primer (5′-TGCATGGTCAGAAGGTATTG-3′) and each of the segment-specific primers listed in Supplementary Table S1. The amplified PCR products were purified and cloned into a pGEM-T Easy vector (Promega). Five clones of each of the 3′ RACE products were sequenced using the Sanger method.

147

148 **Bioinformatic analysis**

149 NCBI ORF finder was used to predict the open reading frames (ORFs) of the cDNA sequences
150 (https://www.ncbi.nlm.nih.gov/orffinder/). Conserved domains of the putatively encoded proteins were
151 predicted using the conserved domain database (CDD)
152 (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). MUSCLE alignment was used to compare the
153 terminal sequences (both 5′ and 3′ terminal) of both viral genome in MEGA-X (Molecular Evolutionary
154 Genetics Analysis) program (Kumar et al., 2018).

155 Deduced amino acid sequences of the viral RdRP and the related previously reported viruses were
156 used for phylogenetic analysis. Online MAFFT version 7 server (https://mafft.cbrc.jp/alignment/server/)
157 was used for sequence alignment [16]. Poorly aligned positions in the alignment were removed using the
158 Gblocks version 0.91b server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) [43]. A
159 maximum-likelihood (ML) tree with a 500 boot strap value, was generated using the MEGA-X. The best
160 fit substitution model (LG+G+I+F) was used for construction of the tree [22].

161

162 **Test for capsidless nature of viral dsRNA**

163 The capsidless nature of HadV1-1NL dsRNA (a replicative form) was tested by RNase A treatment in a
164 crude mycelial homogenate of *F. nygamai* isolate 1NL as previously described [33]. Briefly, a frozen
165 mycelial culture was ground with a mortar and pestle in liquid nitrogen. The mycelial powder was
166 suspended in 0.05 M sodium phosphate and filtered with Miracloth (Merck Millipore). A portion of the
167 mycelial homogenate was treated with 10 µg/mL RNase A for 30 min at 37°C. dsRNA was extracted from
168 the mycelial homogenate before and after the RNase treatment by the cellulose column chromatography
169 method and subjected to agarose gel electrophoresis, as described in the sections above. As a reference, *F.*
170 *oxysporum* isolate A60 harbouring an encapsidated dsRNA virus, Fusarium oxysporum chrysovirus 1 strain
171 A60 (FoCV1-A60), was used. FoCV1-A60 is a novel alphachrysovirus whose detailed properties remain
172 unpublished, but its encapsidated nature was experimentally shown [32, 33].

173

174 **Vertical transmission and effects on colony morphology of HadV1-1NL**

175 Vertical transmission of HadV1-1NL conidia of *F. nygamai* isolate 1NL was analysed according to the
176 previous description [33]. Briefly, conidia (asexual spores) were picked from mycelia (5-day-, 15-day- or

2-month-old) with a pipette and sterilised distilled water. The conidia suspension was diluted and spread on PDA plate to isolate each conidium. Germinated conidial sub-isolates were tested for the presence or absence of HadV1-1NL-RNA1 by the modified mycelial direct RT-PCR method [33, 44] with primers listed in Supplementary Table S1. The reliability of the test was confirmed with five independent conidial sub-isolates from each of the HadV-1NL-RNA1 positive or negative populations by viral dsRNA replicative form profiling and RT-PCR with purified total RNA.

Results

Screening of phytopathogenic fungi for mycoviruses

In 2018, we collected phytopathogenic fungi in Punjab province, Pakistan, and screened them for mycoviruses based on dsRNA accumulation (an indicator of the virus infection) in fungi. Among the screened fungal isolates, an isolate named 1NL was used in this study. The isolate 1NL was identified as *F. nygamai* by sequencing and phylogenetic analyses with ITS (internal transcribed spacer) [29], IGS (intergenic spacer) [27, 31], *ef1a* (elongation factor 1 alpha gene) [8, 30] sequences (Supplementary Fig. S1 and data not shown).

The agarose gel electrophoretic profile of dsRNA purified from *F. nygamai* isolate 1NL resembled that of a Pakistani isolate 7n of *F. oxysporum* isolate, a natural host of HadV1-7n, previously reported by our group [33] (Fig. 1A, left panel). In contrast, the SDS-PAGE profiles of the dsRNA from those fungal isolates were readily distinguishable from each other (Fig. 1A, right panel). The migration of dsRNA elements in agarose gel is based on size, but in polyacrylamide gel it is not necessarily based on size and often influenced by nucleotide sequences and the composition of dsRNA segments [38]. These results implied that the *F. nygamai* isolate 1NL may be infected by a virus with similar genome composition, but it is distinct in nucleotide sequence from the viral strain HadV1-7n.

Fungal colony morphology on PDA plates was distinguishable from the *F. nygamai* isolate 1NL and *F. oxysporum* isolate 7n infected by HadV1-7n [33] (Fig. 1B). *F. nygamai* isolate 1NL grew at a slower rate than *F. oxysporum* isolate 7n. In addition, this *F. nygamai* isolate produced darker pigmentation than *F. oxysporum* 7n.

Molecular and phylogenetic characterisation of HadV1-1NL

To identify the virus harbored in the isolate 1NL of *F. nygamai*, the total RNA fraction extracted from the fungus was subjected to NGS. We screened contigs for sequences similar to known viral sequences using local BLASTN and BLASTX searches. This analysis showed that 10 contigs had significant identity (E-value $\leq 0.0 \sim 10^{-44}$) with the genomic RNA segments of HadV1-7n. However, no significant hit (E-value < 10^{-6}) to HadV1-7n RNA8 in the local BLAST was found. We next determined the terminal sequences of these ten viral contigs using 3′ RLM RACE. The complete sequences of the ten segments ranged from 884 to 2541 nucleotides (nt) in length (Fig. 2A, left). We again performed a BLAST search with the full-length sequences of the novel virus and confirmed that all ten segments showed the highest identity to counterpart segments of HadV1-7n at the nucleotide and amino acid sequence levels (Fig. 2A, Table 1 and Supplementary Table S4). The putative RdRP of the virus showed 89.2% amino acid sequence identity to that of HadV1-7n with a 100% alignment coverage (Table 1). Overall, we conclude that the virus found from the *F. nygamai* isolate 1NL is a novel strain belonging to the same species, "Hadakavirus nanga", as for HadV1-7n and is named "Hadaka virus 1 strain 1NL (HadV1-1NL)".

We numbered the ten genomic segments of HadV1-1NL from RNA1 to RNA10 with a decreasing order of nucleotide length from the longest to the shortest. The complete sequences of these HadV1-1NL genomic segments are available in GenBank/ENA/DDBJ with accession numbers from LC592214 to LC592223, respectively. We showed some properties of the HadV1-1NL genomic segments and hypothetically encoded proteins together with their counterparts of HadV1-7n in Table 1 and Fig. 2. A range of amino acid sequence identity from 49.4% to 92.3% was observed between corresponding proteins of HadV1-1NL and HadV1-7n (Table 1). HadV1-1NL has no segment homologous to HadV1-7n-RNA8, which potentially encodes a putative zinc finger protein (HadV1-P8) (Fig. 2A). HadV1-1NL-RNA1, -RNA2, and -RNA3 putatively encodes an RdRP (P1, RdRP_4, pfam02123), a protein with unknown function (P2), and MTR (P3), respectively, which are conserved between HadV1-7n and polymycoviruses (Fig. 2A and Supplementary Table S4). A domain search on NCBI showed that while the RsmB superfamily (cl33775; 16S rRNA C967 or C1407 C5-methylase) was detected on the HadV1-1NL-RNA3-encoded protein (HadV1-1NL P3), two overlapping MTR-related domains [AdoMet_MTases (cd02440) and RsmB] were found on the counterpart of HadV1-7n-P3 (Fig. 2A and data not shown). Note that HadV1-1NL P1 has GDNQ as the catalytic core residues like polymycoviruses and mononegaviruses (phylum *Negarnaviricota*) in place of the GDD found in the RdRPs of most (+)RNA and dsRNA viruses. Like HadV1-7n, none of the ten HadV1-1NL genomic segments encode PASrp, which is conserved among polymycoviruses that are phylogenetically related to hadakaviruses (Supplementary Table S4). HadV1-1NL-RNA4 to -RNA9 encodes hypothetical proteins of unknown function, which are only known to be conserved among HadV1 strains to date (Fig. 2A and Supplementary Table S4). While HadV1-1NL RNA10 shares 71.2% identity (54% query coverage) to HadV1-7n RNA11 at the nucleotide sequence level, their

largest ORFs could encode 31 (HadV1-1NL P11) and 72 amino acid residues (HadV1-7n P11), respectively, with little sequence identity.

The sequence (5′-CGU----CC(A)-3′) was strictly conserved in all HadV1-1NL segments except for HadV1-1NL RNA6 whose 3′-terminal sequence was 5′-CGU----CGGG-3′ as for its counterpart HadV1-7n RNA7 (Fig. 2B). The similarity in the terminal sequence extends further, particularly between corresponding genome segments of the two HadV1 strains. A minor difference in the extreme 3′-terminal end was observed for several segments between the two virus strains. The extreme 3′-terminal nucleotide "A" was missed or substituted in several genomic segments of HadV1-1NL (Supplementary Fig. S2). It should be noted that 3′-RACE clones were heterogeneous in the 3′-terminal end (see Supplementary Fig. S2). The nucleotide "A" at the 3′-terminus was also absent from some RACE clones of HadV1-7n [33]. A few 3′ RACE clones of HadV1-1NL RNA9 had an additional A residue at the 3′ end. A single 3′ RACE clone of each of HadV1-1NL RNA2 and RNA3 had also an additional A residue at the 3′ end (Supplementary Fig. S2). The consensus 3′-terminal sequence, 5′-CCA-3′ was shared by the HadV1-1NL segments except for HadV1-1NL RNA6 and RNA8 (Fig. 2B)

A phylogenetic tree based on RdRP amino acid sequences showed that HadV1-1NL and HadV1-7n were placed into a clade separated from the clade of polymycoviruses (Fig. 3). Although HadV1 has not been classified to any taxa, this phylogenetic analysis result and the comparison of genome organization (see above) suggest the clear evolutionary separation of HadV1 from the polymycoviruses in the genus *Polymycovirus* in the family *Polymycoviridae*. The RdRP-based evolutionary distance between HadV1-1NL and HadV1-7n was closer than that between two polymycoviruses, Penicillium digitatum polymycovirus 1 and Penicillium janthinellum polymycovirus 1, which are distinct strains of the same polymycovirus species (*Penicillium digitatum polymycovirus 1*) [32]. Thus, it would be reasonable to classify HadV1-7n and HadV1-1NL to the same species.

RNase susceptibility of HadV1-1NL RNAs in mycelial homogenates

To examine whether HadV1-1NL shows capsidless nature like HadV1-7n, the RNase susceptibility of viral replicative dsRNA forms in the mycelial homogenate was tested as described earlier [33]. Briefly, mycelial homogenates in neutral phosphate buffer were treated with RNase A under low salt conditions. The dsRNA profile before and after RNase A treatment was examined. As a reference for RNase A-tolerant dsRNA samples, the mycelial homogenate with an encapsidated dsRNA virus, FoCV1-A60 (an alphachrysovirus), from *F. oxysporum* isolate A-60 was used. After treatment, the dsRNA bands of HadV1-1NL disappeared on an electrophoretic gel in the sample presented with RNase A (Fig. 4). In contrast, the

amount and profile of dsRNA bands of FoCV1-A60 seemed unaltered before and after the RNase treatment (Fig. 4). These results suggest that HadV1-1NL-dsRNA (replicative form) is also a capsidless state, like HadV1-7n-RNAs.

Vertical transmission and asymptomatic infection by Had1-1NL

Our previous study suggests that the vertical transmission rate of HadV1-7n to conidia of *F. oxysporum* isolate 7n decreased as host fungal colonies aged [33]. During attempts to obtain virus-free conidia, we found a similar tendency. Vertical transmission rates of HadV1-1NL to conidia of *F. nygamai* isolate 1NL were examined by mycelial direct RT-PCR with primers detecting HadV1-1NL-RNA1, the RdRP (P1)-encoding segment. The rate of HadV1-1NL transmission through conidia from fresh mycelia (5-day-old) was 83% (n = 31), while that from 15-day-old mycelia was 70% (n = 30) (Supplementary Fig. S3). The transmission rate to conidia from old mycelia (two-month-old) was 0% (n = 15) (Supplementary Fig. S3).

The obtained virus-free and virus-transmitted conidial sub-isolates of *F. nygamai* isolate 1NL were next used to analyse the viral replicative dsRNA profile and to observe host colony growth. Five independent cultures for virus-cured and -transmitted sub-isolates were randomly selected from populations screened by mycelial direct RT-PCR. Consistent with the pre-screening result with RT-PCR, no dsRNA bands nor RT-PCR products of HadV1-1NL-RNA1 were detected in the virus-cured conidial sub-isolates (Fig. 5A). In contrast, all five of the virus-transmitted conidial sub-isolates showed the same dsRNA banding pattern as their parental *F. nygamai* isolate 1NL (Fig. 5A). These results showed an all-or-none transmission of the segments and strongly support the suggestion that all the dsRNA segments are derived from HadV1-1NL.

Comparison of the two groups of these conidial sub-isolates revealed no overt difference in colony morphology on PDA media between the two groups or between them and the parental strain *F. nygamai* 1NL (Fig. 5B). These results suggest asymptomatic infection of the fungal host by the novel hadakavirus strain, HadV1-1NL.

Discussion

In this paper, we characterised a second hadakavirus termed hadaka virus 1-1NL (HadV1-1NL), which was isolated from *F. nygamai* infecting the leaves of a pomegranate plant in Pakistan. HadV1-1NL is a close relative of the first described hadakavirus HadV1-7n [33]. Comparative analyses of the two mycovirus strains showed interesting similarities and differences. The similarities include amino acid

303 sequences between their protein counterparts (49.4–92.2% identity) (Table 1), conserved terminal sequence,
304 lack of the PASrp-encoding segment, susceptibility to RNase A, and profiles for vertical transmission via
305 conidia. The shared molecular and phylogenetic characteristics support our above-mentioned proposal that
306 the two mycoviruses belong to the same species, "Hadakavirus nanga", and show that hadakaviruses are
307 distinct from polymycoviruses within the family *Polymycoviridae*. Interesting differences between the two
308 strains are the genome segment number, with 10 for HadV1-1NL vs. 11 for HadV1-7n, and the coding
309 capacity of the smallest segment, 33 amino acids (HadV1-1NL P11) for HadV1-1NL RNA10 vs. 72 amino
310 acids (HadV1-7n P11) for HadV1-7n RNA11 (Table 1). Different genome segment numbers within single
311 genera or families are rarely found. Examples include mycoreoviruses (family *Reoviridae*) with either 11
312 or 12 dsRNA genomic segments [41, 47], chrysoviruses (family *Chrysoviridae*) with three to seven dsRNA
313 genomic segments [21], and polymycoviruses with four to eight RNA segments [15, 20]. Whether these
314 small proteins encoded by HadV1-1NL RNA10 and HadV1-7n RNA11 are expressed remains elusive, as
315 there are many mini-cistrons in their 5′-untranslated regions preceding the small ORFs (data not shown).

316 The recent characterisation of many peculiar mycoviruses has contributed to enhance our
317 understanding of virus diversity and evolution [4, 5, 28, 37]. Peculiar mycoviruses include capsidless multi-
318 and non-segmented (+)RNA viruses that are categorised into five groups. The first group is represented by
319 hypoviruses (picornavirus-like supergroup; phylum *Pisuviricota*) and endornaviruses (alpha/flavivirus-like
320 supergroup; phylum *Kitrinoviricota*) whose replicative dsRNA form is encased in host-derived
321 membranous vesicles [42, 45]. The second group is exemplified narnaviruses (levivirus-like supergroup;
322 phylum *Lenarviricota*) with genomic RNA associated with their RdRP molecules [49]. The third group is
323 polymycoviruses (picornavirus-like supergroup), the genomic dsRNA of which is associated with their
324 PASrp [15, 20]. It should be noted that polymycoviruses, although phylogenetically related to caliciviruses
325 with (+)RNA genomes, are classified as dsRNA viruses due to their infectivity as purified dsRNA or
326 PASrp-associated dsRNA. Group IV includes yadokariviruses (picornavirus-like supergroup) which
327 highjack the capsids of partner dsRNA toti-like viruses (branch 4 supergroup, phylum *Duplornaviricota*)
328 [12, 53]. Group V accommodates hadakaviruses (picornavirus-like supergroup) that are phylogenetically
329 related to polymycoviruses that likely exist as a naked form accessible by RNase A at least in mycelial
330 homogenates [33]. Hadaka virus 1 was named after its capsidless nature; "Hadaka" is a Japanese word
331 meaning "naked" [33]. Hadakaviruses are phylogenetically closely related to polymycoviruses when RdRP
332 amino acid sequences were analysed. However, hadakaviruses do not possess PASrps, which characterise
333 all known polymycoviruses, and their genomic RNAs do not appear to be tightly associated with proteins
334 in mycelial homogenates. This extremely unusual property was first demonstrated for HadV1-7n, while this
335 study confirmed the capsidless nature of another hadakavirus strain, HadV1-1NL. It is noteworthy that this
336 Hadaka nature is different from that of other previously reported capsidless viruses. As discussed above,

polymycoviruses are tightly associated with PASrps to form RNA-protein complexes or filamentous particles and are pelleted by ultracentrifugation. Narnaviruses and likely mitoviruses (mitochondrially-replicating viruses) are associated with their sole gene products RdRPs. Hypoviruses and endornaviruses are encased by host-derived membrane vesicles and these viruses are accessed by RNase A only after treatment with detergent. Hadakaviruses are not pelleted by ultracentrifugation and are accessible by nuclease without detergent treatment. The hadaka RNA nature needs to be further explored.

Determination of the taxonomical positions of capsidless viruses is not as simple as encapsidated viruses for which genomic nucleic acids in virions are readily identifiable. In the case of hypoviruses, they initially used to be classified as dsRNA viruses based on the high accumulation of viral derived dsRNA (these dsRNA molecules are currently considered as a virus replicative form) contained in Golgi-derived vesicles [11]. However, based on their close phylogenetic and evolutionary relationship to the expanded picorna-like supergroup with (+)RNA genomes and the infectivity of hypovirus transcripts, hypoviruses are now classified as (+)RNA viruses [42]. In the case of polymycoviruses, they show phylogenetic affinity to caliciviruses (order *Picornavirales, Pisuviricota*) and astroviruses (order *Stellavirales, Pisuviricota*) with (+)RNA genomes rather than partitiviruses with dsRNA genomes within the phylum *Duplornaviricota*. However, because their dsRNA, whether deproteinised or associated with PASrps, is infectious, polymycoviruses are classified as a dsRNA viruses [46]. The greater phylogenetic affinity of hadakaviruses to animal caliciviruses and astroviruses within the expanded picorna-like supergroup with (+)RNA genomes allowed Sato and others to propose that HadV1 is regarded as a (+)RNA virus [33]. This study also strengthens Sato's proposal. Hadakaviruses are phylogenetically closely related to but considerably distinct from polymycoviruses [33] (Fig. 2). Furthermore, the differences in PASrp coding capacity, RNase susceptibility, and sedimentation profile in CsCl and sucrose density gradient centrifugation are sufficient to classify the two virus groups into different families. We herein propose the creation of a new family "Hadakaviridae" that accommodates two hadakaviruses (HadV1-7n and HadV1-1NL) from different *Fusarium* spp. in Pakistan.

Fusarium spp. include many important phytopathogens, including *F. graminearum* and *F. oxysporum*. There are several research groups that have conducted virus hunting with *Fusarium* spp. [6, 23, 24, 26]. These studies discovered many different viruses spanning (+)RNA viruses, (-)RNA viruses, dsRNA viruses and ssDNA viruses. Some of them were well-characterised, including Fusarium graminearum virus 1 (a capsidless fusarivirus related to hypoviruses) [35, 51], Fusarium graminearum virus China 9 (a betachrysovirus) [3], and Fusarium graminearum gemytripvirus 1 (a genomovirid, ssDNA virus) [25]. However, no hadakaviruses had been reported until the 2020 study by Sato and others [33]. Hadakaviruses have been isolated only from *Fusarium* spp. in Pakistan thus far. An additional hadakavirus was detectable

370 from an Ethiopian strain of *Fusarium* spp. (Sotaro Chiba at Nagoya University, personal communication).
371 These suggest that hadakaviruses exist, although may not be prevalent, at least in *Fusarium* spp. from the
372 India Subcontinent and Africa.

373 HadV1-1NL causes asymptomatic infection in the original host fungal species *F. nygamai* as long
374 as tested on nutrition-rich PDA. This conclusion is solidly based on a comparison of multiple isogenic
375 HadV1-1NL-infected and -free fungal isolates, the second of which was obtained from single spore
376 isolation (Fig. 5 and Supplementary Fig. S3). However, whether HadV1-1NL shows symptomatic or
377 asymptomatic infection under natural conditions remains an open question. Another interesting biological
378 finding was a difference in vertical transmission rate between spores produced in old and young colonies.
379 Previously, HadV1-7n vertical transmission rates in *F. oxysporum* were reported to vary depending on the
380 age of mycelia that formed spores [33]. This phenomenon was confirmed with a different HadV1 (1NL)
381 strain and its fungal host, *F. nygamai*, in this study. It remains unclear how the vertical transmission of
382 HadV1-1NL is governed. This difference may be related to virus contents in spores, as in the case for the
383 prototype hypovirus CHV1-EP155 [39]. It should be noted that these biological properties, symptomless
384 infection and different age-dependent vertical transmission rates are shared by the other hadakavirus strain,
385 HadV1-7n, despite the difference in host fungi.

386

387 **Supplementary Information**

388 The online version contains supplemen- tary material available a

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393 **Author contributions**

394 Conceptualization: N. S., Investigation; H. A. K., Y. S., A. J., H. K., N. S. Supervision: A. J., M. F. B.
395 Writing - original draft: H. A. K., Y. S., N. S. Writing – reviewing & editing: A. J., M. F. B., H. K. Funding
396 acquisition: N. S.

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Compliance with ethical standards

Conflicts of interest

The authors declare that there are no conflicts of interest.

Human and animal rights

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

Figure legends

Fig. 1. Viral dsRNA profile and colony morphology of three fungal isolates. Two fungal isolates (1NL-VF and 1NL) of *Fusarium nygamai* (*Fn*) and an isolate (7n) of *Fusarium oxysporum* (*Fo*) were used. The isolate 1NL is infected by a novel virus, HadV1-7n, while the isolate 1NL-VF is a virus-free conidial sub-isolate (see Fig. 5) of 1NL. (A) The electrophoretic profile of viral dsRNA on 1% agarose gel (left panel) or 10% SDS-polyacrylamide gel (right panel). Lane "M" shows the genomic dsRNA profile of mycoreovirus 1/S10ss as size marker. (B) Seven-day-old fungal colonies cultured on PDA media.

Fig. 2. Comparison of genome organisation between HadV1-1NL and HadV1-7n. (A) Schematic representation of HadV1-1NL and HadV1-7n genome organisation. The arrows indicate the pairs of homologous segments conserved between HadV1-1NL and HadV1-7n (see also Table 1). The black boxes indicate the longest open reading frame (ORF) for each segment. "PmV/HadV1-P*n*" indicate ORFs that encode a homolog of the counterparts conserved among polymycoviruses and HadV1-7n. "HadV1-P*n*" indicate ORFs that encode a homolog of the counterparts specific to HadV1-7n. HadV1-1NL P11 and HadV1-7n P11 are HadV1-strain-specific hypothetical proteins that are encoded by the longest ORFs on HadV1-1NL RNA10 or HadV1-7n RNA11, respectively. Colored boxes inside black boxes represent position of conserved domains [RNA_dep_RNAP (RNA-dependent RNA polymerase, cd01699), RsmB

(16S rRNA C967 or C1407 C5-methylase, RsmB/RsmF family, cl33775), and AdoMet_MTases (S-adenosylmethionine-dependent methyltransferases, cd02440)] or a motif (C_2H_2 Zn finger). (B) Multiple sequence alignment of 5'- and 3'-terminals of HadV1-1NL and HadV1-7n genomic segments. The sequence alignment was visualised using MEGA-X software. The pairs of homologous segments are connected with lines attached with arrows.

Fig. 3. Phylogenetic relationship of hadakaviruses (HadV1-1NL and HadV1-7n) and polymycoviruses (members in the genus *Polymycovirus* in the family *Polymycoviridae*) based on the amino acid sequence of RdRP. The maximum likelihood tree (model LG+G+I+F) was generated using the MEGA-X tool. Accession numbers are shown in the tree. The values next to the branches indicate bootstrap probability with 500 replicates. Branch length indicates the number of amino acid substitutions per site. HadV1-1NL and HadV1-7n were indicated with filled or open arrows, respectively.

Fig. 4. RNase A susceptibility of viral dsRNA in mycelial homogenates. Agarose gel electrophoretic profiles of viral dsRNA extracted before (-) and after (+) RNaseA treatment are shown. HadV1-1NL carried in *Fusarium nygamai* isolate 1NL (*Fn*-1NL) and Fusarium oxysporum chrysovirus 1 strain A60 (FoCV1-A60) harboured in *Fusarium oxysporum* isolate A60 (*Fo*-A60) were tested. FoCV1-A60 is an alphachrysovirus, multi-segmented encapsidated dsRNA virus. The lane "M" shows the genomic dsRNA profile of mycoreovirus 1/S10ss as a size marker.

Fig. 5. Vertical transmission of HadV1-1NL to conidia in *F. nygamai* isolate 1NL. Five independent conidial sub-isolates for each HadV1-1NL-free [HadV1(-)] and HadV1-1NL-transmitted [HadV1(+)] groups were used. (A) Agarose gel electrophoretic profiles of dsRNA (top panel) and RT-PCR products (middle and bottom panels). HadV1-1NL-RNA1 (the RdRP-encoding segment) and host *ef1α* gene were detected by the RT-PCR using a total RNA template. Lane "M" shows the genomic dsRNA profile of mycoreovirus 1/S10ss as a size marker. (B) Fungal colonies cultured on PDA media.

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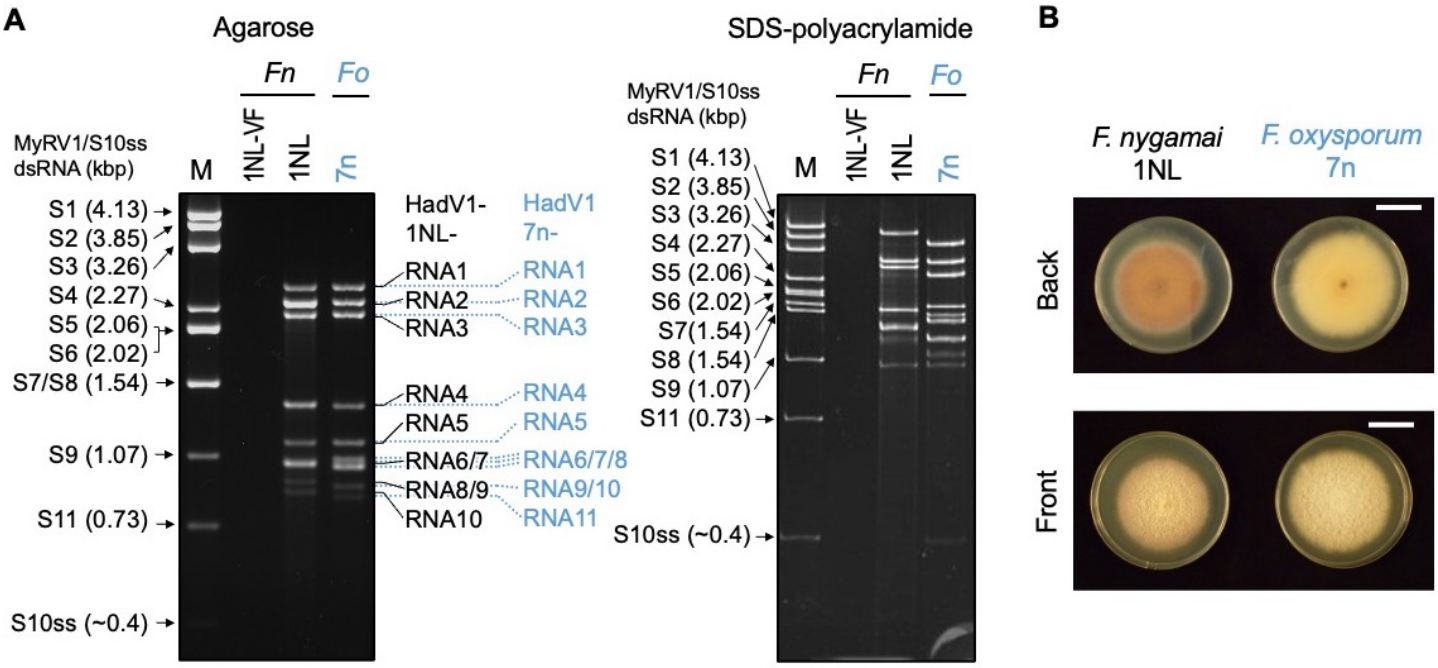
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Fo
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M
1NL-VF
1NL
7n
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S2 (3.85)
S3 (3.26)
S4 (2.27)
S5 (2.06)
S6 (2.02)
S7/S8 (1.54)
S9 (1.07)
S11 (0.73)
S10ss (~0.4)
S7(1.54)
S8 (1.54)
HadV1-1NL-
HadV1 7n-
RNA1
RNA2
RNA3
RNA4
RNA5
RNA6/7
RNA8/9
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RNA9/10
RNA11
B
F. nygamai 1NL
F. oxysporum 7n
Back
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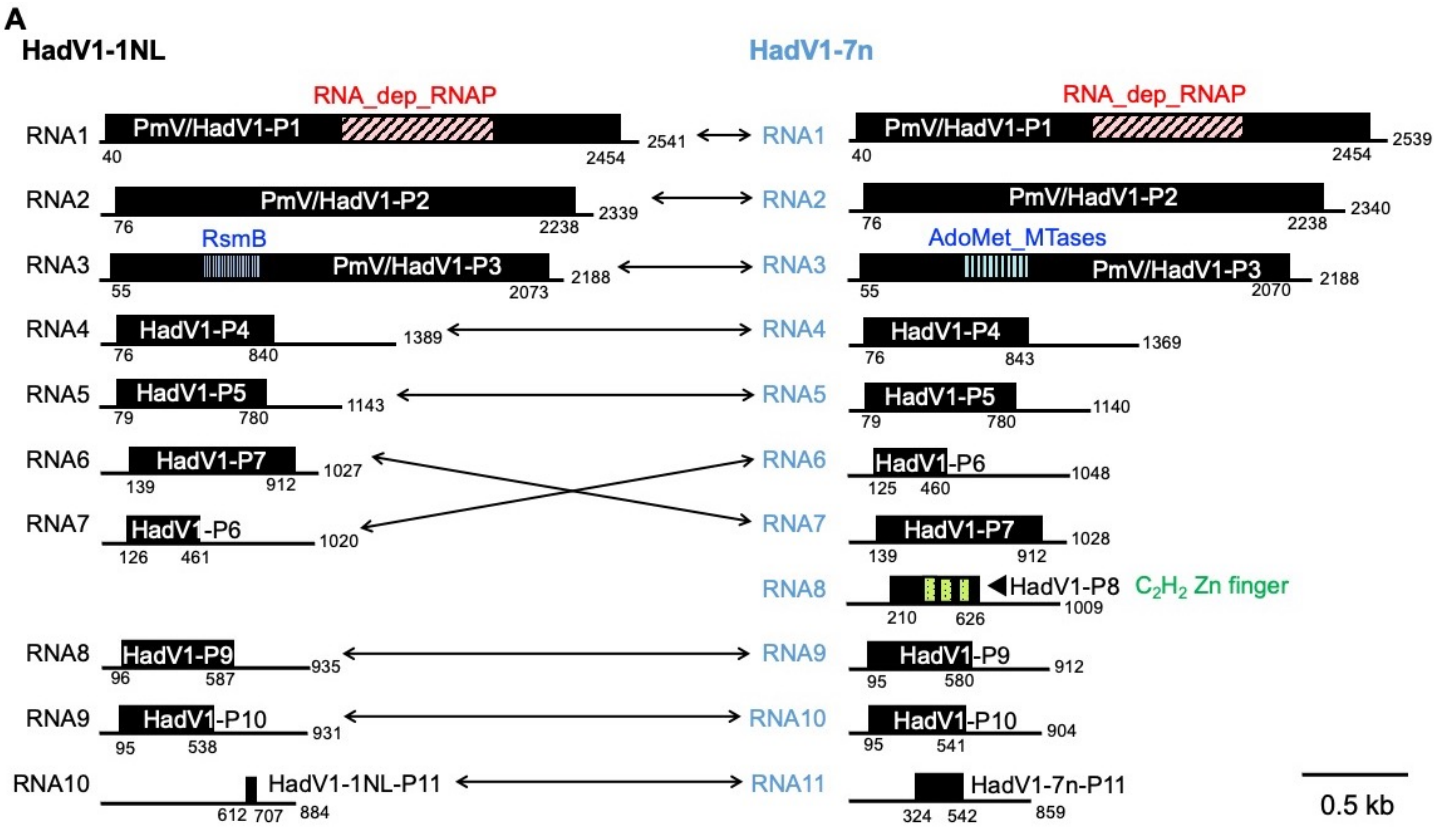
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RNA2
PmV/HadV1-P2
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RNA2
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2238
2340
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AdoMet_MTases
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2188
RNA3
PmV/HadV1-P3
55
2070
2188
RNA4
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RNA4
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1369
RNA5
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RNA5
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79
780
1140
RNA6
HadV1-P7
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RNA6
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1048
RNA7
HadV1-P6
126
461
1020
RNA7
HadV1-P7
139
912
1028
RNA8
HadV1-P8
C2H2 Zn finger
210
626
1009
RNA8
HadV1-P9
96
587
935
RNA9
HadV1-P9
95
580
912
RNA9
HadV1-P10
95
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95
541
904
RNA10
HadV1-1NL-P11
612
707
884
RNA11
HadV1-7n-P11
324
542
859
0.5 kb
B
5' terminal
3' terminal
HadV1-1NL-RNA1
HadV1-7n-RNA1
HadV1-1NL-RNA2
HadV1-7n-RNA2
HadV1-1NL-RNA3
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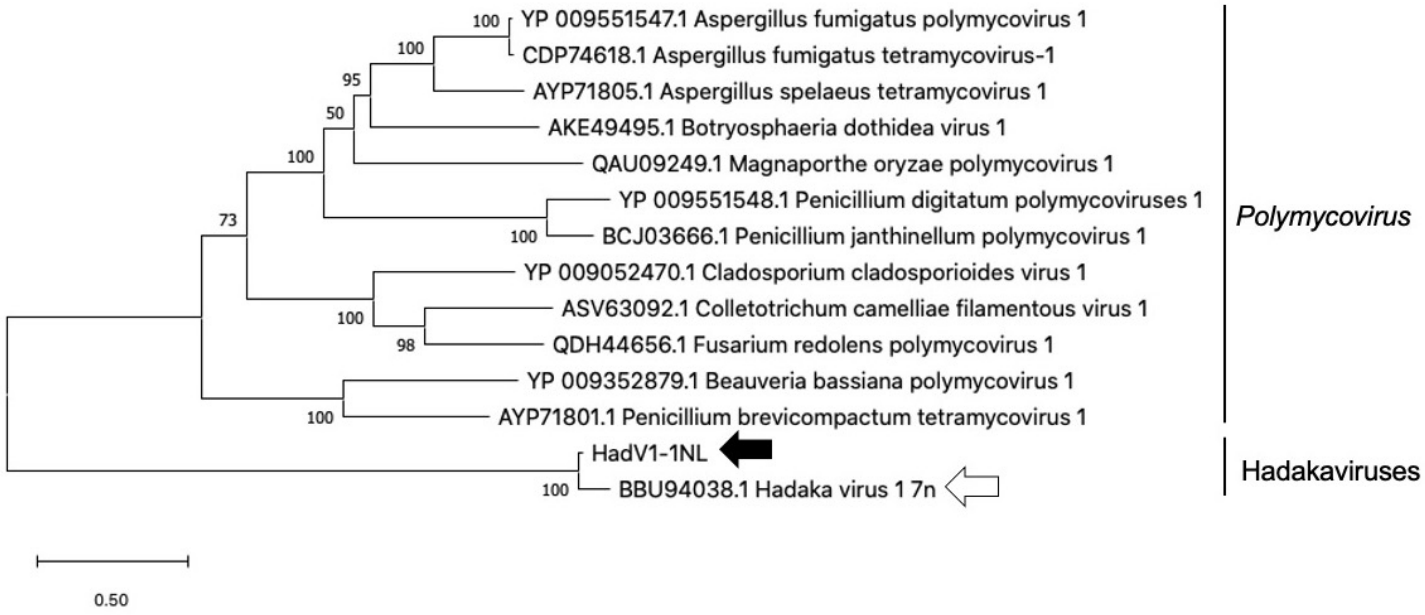
RdRP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73
100
BCJ03666.1 Penicillium janthinellum polymycovirus 1
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98
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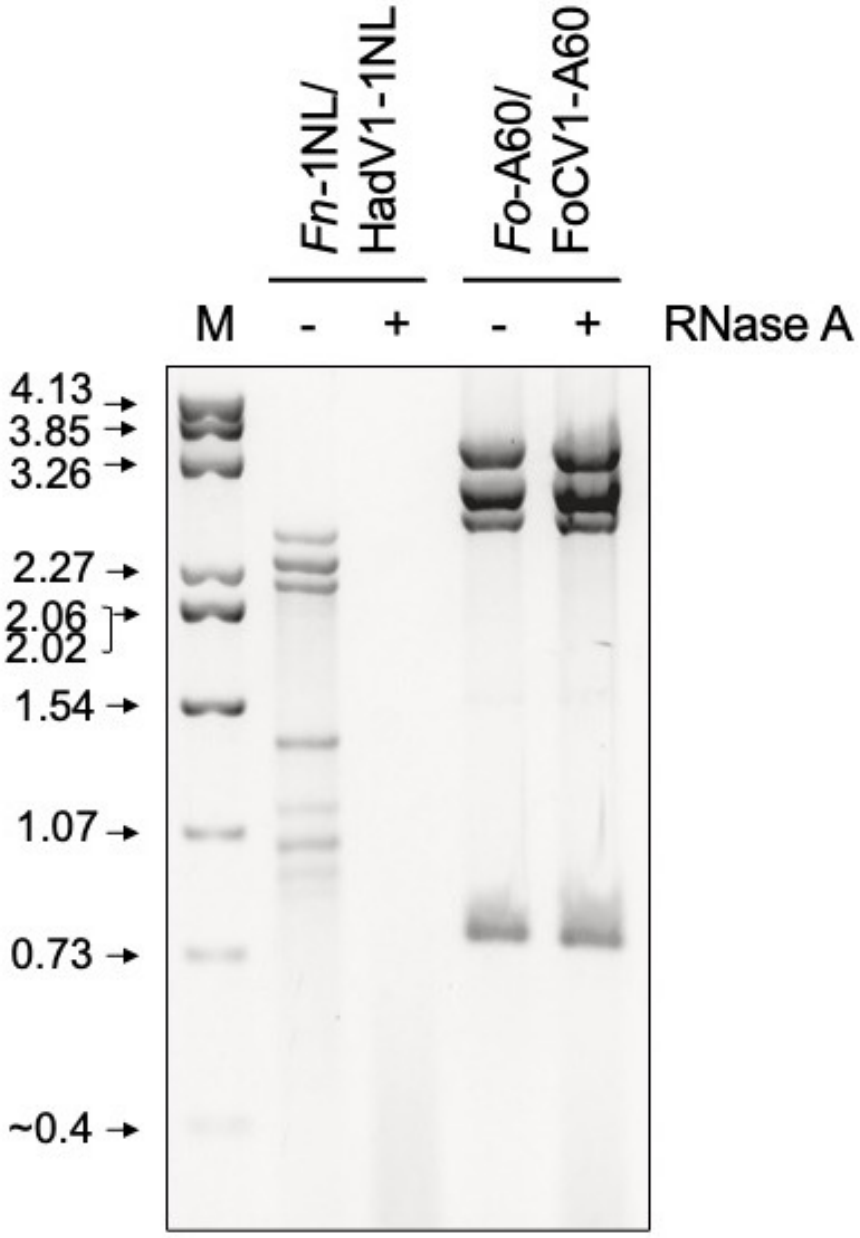
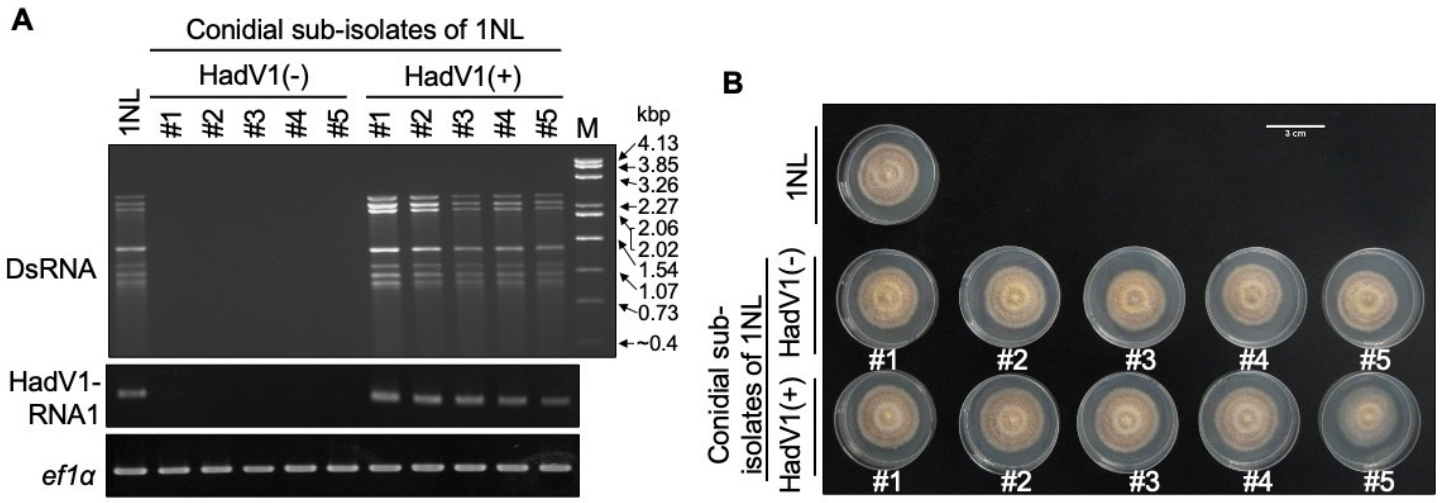

Fn-1NL/
HadV1-1NL
Fo-A60/
FoCV1-A60
M
-
+
-
+
RNase A
4.13
3.85
3.26
2.27
2.06
2.02
1.54
1.07
0.73
~0.4


A
Conidial sub-isolates of 1NL
HadV1(-)
HadV1(+)
1NL
#1
#2
#3
#4
#5
#1
#2
#3
#4
#5
M
kbp
4.13
3.85
3.26
2.27
2.06
2.02
1.54
1.07
0.73
~0.4
DsRNA
HadV1-RNA1
ef1α
B
Conidial sub-isolates of 1NL
1NL
HadV1(-)
HadV1(+)
3 cm
#1
#2
#3
#4
#5
#1
#2
#3
#4
#5

Supplementary Data for

A second capsidless hadakavirus strain with a 10 positive-sense single-stranded RNA genomic segments from *Fusarium nygamai*

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Supplementary figures, S1 to S3.

Supplemental tables, 4.

Legends to Supplementary Figures

Fig. S1. Phylogenetic positions of the fungal isolates 1NL and 7n in *Fusarium* species. The phylogenetic trees were constructed based on the nucleotide sequence of the intergenic spacer (IGS) (A) or translation elongation factor 1 alpha gene (*ef1α*) (B). The maximum likelihood tree based on the best fit model [Hasegawa-Kishino-Yano (HKY)] was generated using the MEGA-X tool. The values next to the branches indicate bootstrap probability with 1000 replicates. Isolates or strains of *F. oxysporum*, *F. nygamai*, and *F. mangiferae* listed in Table S2 and Table S3 were involved in the analyses together with the isolates 1NL and 7n. The isolate 7n was previously identified as *F. oxysporum* (Sato et al., 2020). Isolates 1NL and 7n are indicated by filled or open arrows, respectively.

Fig. S2. Sequence alignment of 3′-RACE clones. RLM-RACE was carried out to determine the 3′-terminal sequences of the genomic RNA of HadV1-1NL as described in the Materials and Methods. The sequences of obtained clones, at least five clones for each segment, were aligned. All RACE clones for the identification of 3′-terminal sequences of HadV1-1NL genomic segments. Thirteen or fourteen nucleotide sequences at 3′-terminal of all HadV1-1NL genomic segments were shown. The clones indicated by a red bracket were representatively shown in Fig. 2 and deposited with the public databases.

Fig. S3. Vertical transmission rate of HadV1-1NL to conidia in *F. nygamai* isolate 1NL. Sub-isolates each derived from single conidia isolated from 5-day-old (5-do), 15-day-old (15-do), and 2-month-old (2-mo) mycelia of *F. nygamai* isolate 1NL were used for the analysis. (A) Detection of the presence or absence of HadV1-1NL-RNA1 (the RdRP-encoding segment) by RT-PCR. “NC” and “PC” indicate negative or positive controls, respectively. Lane “M” contains the GeneRuler 1kb DNA Ladder (Thermo Fisher Scientific Inc.). (B) The transmission rate of HadV1-1NL to the conidial sub-isolates. The percentage was calculated based on results in (A). The percentage of HadV1-1NL-transmitted [HadV1(+)] and HadV1-1NL-free [HadV1(-)] lines was represented with dark or light grey boxes, respectively.

Fig. S1

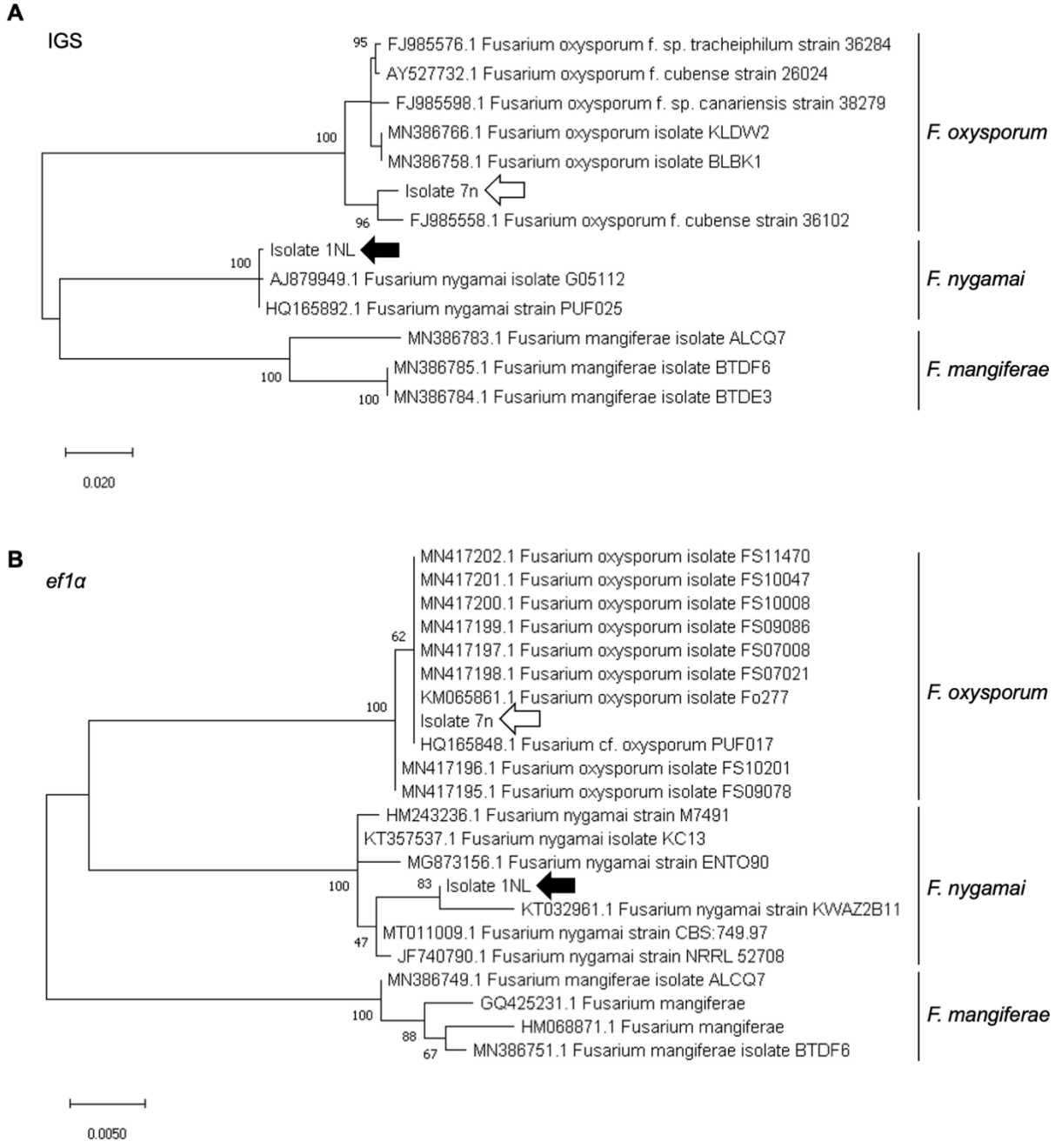
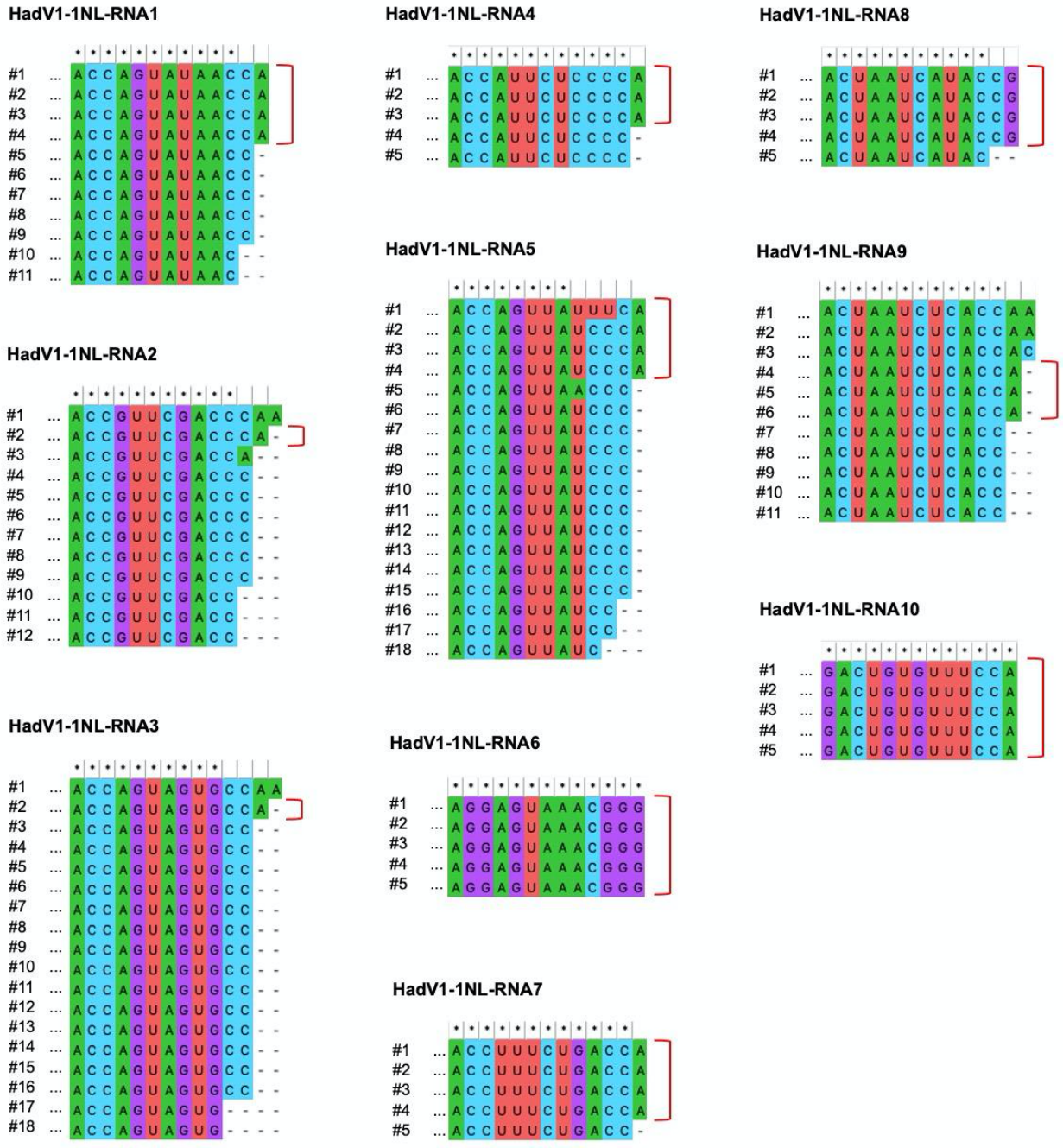

A
IGS
FJ985576.1 Fusarium oxysporum f. sp. tracheiphilum strain 36284
AY527732.1 Fusarium oxysporum f. cubense strain 26024
FJ985598.1 Fusarium oxysporum f. sp. canariensis strain 38279
MN386766.1 Fusarium oxysporum isolate KLDW2
MN386758.1 Fusarium oxysporum isolate BLBK1
Isolate 7n
FJ985558.1 Fusarium oxysporum f. cubense strain 36102
Isolate 1NL
AJ879949.1 Fusarium nygamai isolate G05112
HQ165892.1 Fusarium nygamai strain PUF025
MN386783.1 Fusarium mangiferae isolate ALCQ7
MN386785.1 Fusarium mangiferae isolate BTDF6
MN386784.1 Fusarium mangiferae isolate BTDE3
95
100
96
100
100
100
F. oxysporum
F. nygamai
F. mangiferae
0.020
B
ef1α
MN417202.1 Fusarium oxysporum isolate FS11470
MN417201.1 Fusarium oxysporum isolate FS10047
MN417200.1 Fusarium oxysporum isolate FS10008
MN417199.1 Fusarium oxysporum isolate FS09086
MN417197.1 Fusarium oxysporum isolate FS07008
MN417198.1 Fusarium oxysporum isolate FS07021
KM065861.1 Fusarium oxysporum isolate Fo277
Isolate 7n
HQ165848.1 Fusarium cf. oxysporum PUF017
MN417196.1 Fusarium oxysporum isolate FS10201
MN417195.1 Fusarium oxysporum isolate FS09078
HM243236.1 Fusarium nygamai strain M7491
KT357537.1 Fusarium nygamai isolate KC13
MG873156.1 Fusarium nygamai strain ENTO90
Isolate 1NL
KT032961.1 Fusarium nygamai strain KWAZ2B11
MT011009.1 Fusarium nygamai strain CBS:749.97
JF740790.1 Fusarium nygamai strain NRRL 52708
MN386749.1 Fusarium mangiferae isolate ALCQ7
GQ425231.1 Fusarium mangiferae
HM068871.1 Fusarium mangiferae
MN386751.1 Fusarium mangiferae isolate BTDF6
62
100
100
83
47
100
88
67
F. oxysporum
F. nygamai
F. mangiferae
0.0050

Fig. S2



58
59

60

Fig. S3

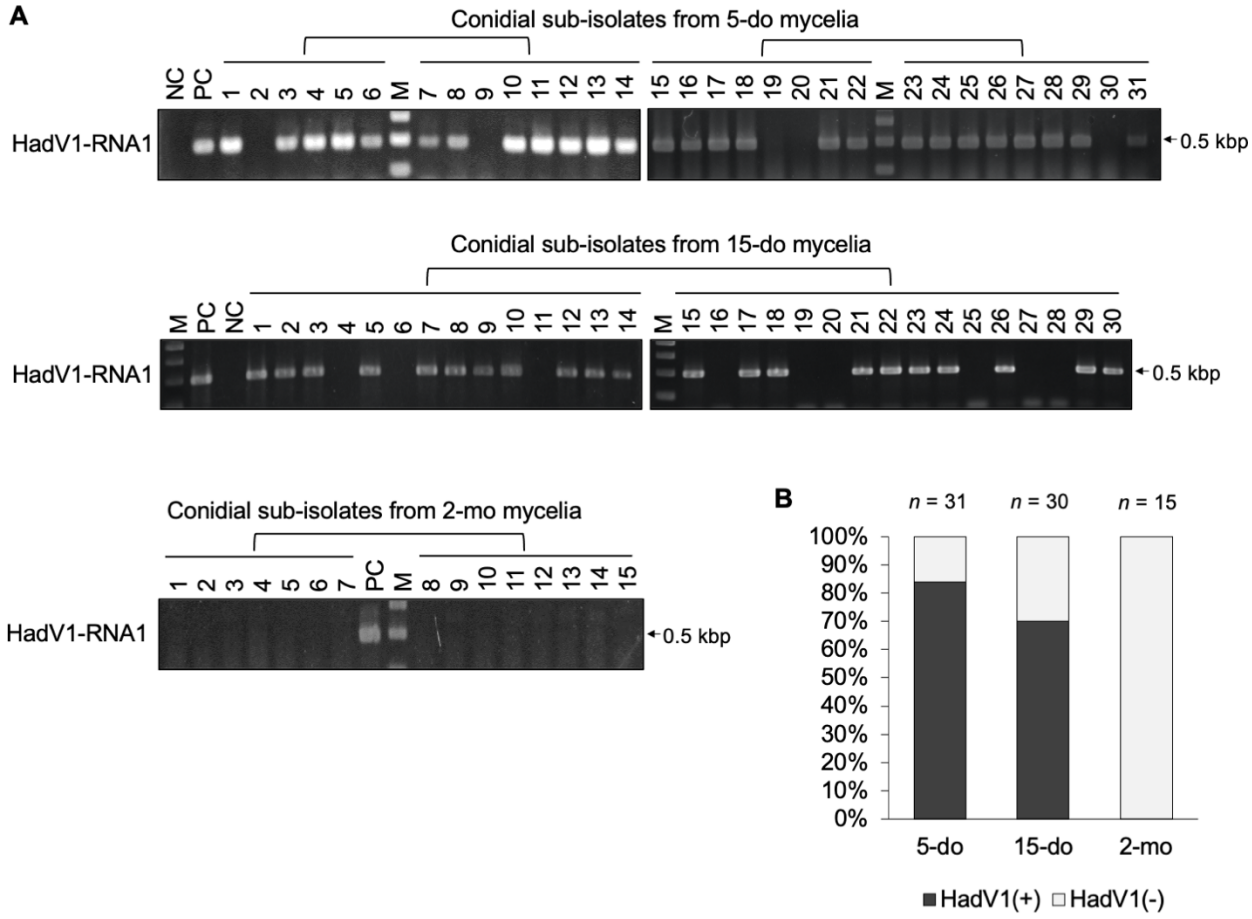

A
Conidial sub-isolates from 5-do mycelia
NC PC 1 2 3 4 5 6 M 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 M 23 24 25 26 27 28 29 30 31
HadV1-RNA1
0.5 kbp
Conidial sub-isolates from 15-do mycelia
M PC NC 1 2 3 4 5 6 7 8 9 10 11 12 13 14 M 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
HadV1-RNA1
0.5 kbp
Conidial sub-isolates from 2-mo mycelia
1 2 3 4 5 6 7 PC M 8 9 10 11 12 13 14 15
HadV1-RNA1
0.5 kbp
B
n = 31
n = 30
n = 15
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
5-do
15-do
2-mo
HadV1(+)
HadV1(-)

Table S1. Primers used in this study.

For	Target	Forward primer sequence (5'→3')	Reverse primer sequence (5'→3')
RACE	5'-terminal of HadV1-1NL-RNA1		GCAACCAAATCATCAGGACACTT
	3'-terminal of HadV1-1NL-RNA1	ACAATTCGATCGGAGAGGTAGAT	
	5'-terminal of HadV1-1NL-RNA2		CACCTGACCTTTCTTAAGACTG
	3'-terminal of HadV1-1NL-RNA2	AAAGGAGGAGGATCATGGATATG	
	5'-terminal of HadV1-1NL-RNA3		AACCCTCCTATTAGTGACAAGC
	3'-terminal of HadV1-1NL-RNA3	GGTGCCTACGGTAATTTTGTCAA	
	5'-terminal of HadV1-1NL-RNA4		GGAAGTGACTGACATCCTCAA
	3'-terminal of HadV1-1NL-RNA4	ATTCACCACTTACCTTGGCCA	
	5'-terminal of HadV1-1NL-RNA5		GTAACATCCTTGGTTGGCAATG
	3'-terminal of HadV1-1NL-RNA5	TAAGGACATCGGGGAGCTCAA	
	5'-terminal of HadV1-1NL-RNA6		AACGACGGACATAGGCCCAT
	3'-terminal of HadV1-1NL-RNA6	GATTGTTAGAGCAGCGGAAGA	
	5'-terminal of HadV1-1NL-RNA7		CGAGATTGGACCTACTAGACATA
	3'-terminal of HadV1-1NL-RNA7	TATGTCTAGTAGGTCCAATCTCG	
	5'-terminal of HadV1-1NL-RNA8		GAGAGTTCTCCTACACGAATAAG
	3'-terminal of HadV1-1NL-RNA8	ATGTCATCTATGCTCGAGCAGA	
	5'-terminal of HadV1-1NL-RNA9		TATTCCATGACTCCCTTTGCGAA
	3'-terminal of HadV1-1NL-RNA9	TTCGCAAAGGGAGTCATGGAATA	
	5'-terminal of HadV1-1NL-RNA10		TATTCGGAGGTACTGTCACCA
	3'-terminal of HadV1-1NL-RNA10	GTCGCAATTCTCTTCTTGAACG	
Fungal identification	ITS	TCCGTAGGTGAACCTGCGG (ITS1)	TCCTCCGCTTATTGATATGC (ITS4)
	IGS	GTAAGCCGTCCTTCGCCTCG (FIGS11)	GCAAAATTCAATAGTATGGC (FIGS12)
	ef1α	ATGGGTAAGGARGACAAGAC (EF-1)	GGARGTACCAGTSATCATGTT (EF-2)
RT-PCR	HadV1-1NL-RNA1	AAGAGGAAAGACCCCGAGTTG	TTGGACTTGTCAACGAGCCTCT

Table S2. Accession numbers for internal genomic spacer (IGS) sequences of *Fusarium* species involved in the phylogenetic analysis.

***Fusarium* species and isolate/strain**	**Accession number**	**Reference**
Fusarium nygamai strain PUF025	HQ165892.1	(Wang et al., 2011)
Fusarium nygamai isolate G05112	AJ879949.1	(Mirete et al., 2013)
Fusarium oxysporum f. sp. *cubense* strain 36102	FJ985558.1	(O'Donnell et al., 2009)
Fusarium oxysporum f. sp. *tracheiphilum* strain 36284	FJ985576.1	(O'Donnell et al., 2009)
Fusarium oxysporum f. sp. *canariensis* strain 38279	FJ985598.1	(O'Donnell et al., 2009)
Fusarium oxysporum f. sp. *cubense* strain 26024	AY527732.1	(O'Donnell et al., 2004)
Fusarium oxysporum isolate KLDW2	MN386766.1	(Kee et al., 2020)
Fusarium oxysporum isolate BLBK1	MN386758.1	(Kee et al., 2020)
Fusarium mangiferae isolate ALCQ7	MN386783.1	(Kee et al., 2020)
Fusarium mangiferae isolate BTDE3	MN386784.1	(Kee et al., 2020)
Fusarium mangiferae isolate BTDF6	MN386785.1	(Kee et al., 2020)

Table S3. Accession numbers for translation elongation factor 1 alpha gene (*elfa*) sequences of *Fusarium* species used in the phylogenetic analysis.

***Fusarium* species and isolate/strain**	**Accession number**	**Reference**
Fusarium nygamai strain CBS:749.97	MT011009.1	(Yang et al., 2020)
Fusarium nygamai strain M7491	HM243236.1	(Amatulli et al., 2012)
Fusarium nygamai isolate KC13	KT357537.1	(Bogner et al., 2016)
Fusarium nygamai strain ENTO90	MG873156.1	(Petrovic et al., 2013)
Fusarium nygamai strain KWAZ2B11	KT032961.1	(Pili et al., 2016)
Fusarium nygamai strain NRRL 52708	JF740790.1	(O'Donnell et al., 2012)
Fusarium cf. *oxysporum* PUF017	HQ165848.1	(Wang et al., 2011)
Fusarium oxysporum isolate FS11470	MN417202.1	(Duan et al., 2020)
Fusarium oxysporum isolate FS10047	MN417201.1	(Duan et al., 2020)
Fusarium oxysporum isolate FS10008	MN417200.1	(Duan et al., 2020)
Fusarium oxysporum isolate FS09086	MN417199.1	(Duan et al., 2020)
Fusarium oxysporum isolate FS07021	MN417198.1	(Duan et al., 2020)
Fusarium oxysporum isolate FS07008	MN417197.1	(Duan et al., 2020)
Fusarium oxysporum isolate Fo277	KM065861.1	(Koyyappurath et al., 2015)
Fusarium oxysporum isolate FS10201	MN417196.1	(Duan et al., 2020)
Fusarium oxysporum isolate FS09078	MN417195.1	(Duan et al., 2020)
Fusarium mangiferae	HM068871.1	(Zhan et al., 2012)
Fusarium mangiferae isolate BTDF6	MN386751.1	(Kee et al., 2020)
Fusarium mangiferae isolate ALCQ7	MN386749.1	(Kee et al., 2020)
Fusarium mangiferae	GQ425231.1	(Pinaria et al., 2010)

91 **Table S4. Results of BLASTX search with HadV1-1NL.**

Query	Description	Scientific Name	Genus	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
HadV1-1NL-RNA1	RNA-dependent RNA polymerase [Hadaka virus 1]	Hadaka virus 1	Unclassified (Hadakavirus)	1477	1477	94%	0	89.18	804	BBU94038.1
	putative RNA-dependent RNA polymerase [Magnaporthe oryzae polymycovirus 1]	Magnaporthe oryzae polymycovirus 1	*Polymycovirus*	287	287	75%	6.00E-81	33.33	766	QAU09249.1
	RNA-dependent RNA polymerase [Aspergillus fumigatus polymycovirus 1]	Aspergillus fumigatus polymycovirus 1	*Polymycovirus*, unassigned isolate	284	284	68%	6.00E-80	32.26	763	YP_009551547.1
	RNA dependent RNA polymerase [Aspergillus fumigatus tetramycovirus-1]	Aspergillus fumigatus tetramycovirus-1	*Polymycovirus*	284	284	68%	1.00E-79	32.43	763	CDP74618.1
	RNA dependent RNA polymerase [Beauveria bassiana polymycovirus 1]	Beauveria bassiana polymycovirus 1	*Polymycovirus*	281	281	75%	2.00E-78	31.76	775	YP_009352879.1
	RNA-dependent RNA polymerase [Botryosphaeria dothidea virus 1]	Botryosphaeria dothidea virus 1	*Polymycovirus*	275	275	68%	1.00E-76	32.43	757	YP_009342446.1
	RNA-dependent RNA polymerase [Penicillium brevicompactum tetramycovirus 1]	Penicillium brevicompactum tetramycovirus 1	*Polymycovirus*	275	275	76%	3.00E-76	29.73	775	AYP71801.1
	RNA-dependent RNA polymerase [Botryosphaeria dothidea virus 1]	Botryosphaeria dothidea virus 1	*Polymycovirus*, unassigned isolate	270	270	68%	6.00E-75	32.71	758	ALZ41794.1
	putative RNA-dependent RNA polymerase [Cladosporium cladosporioides virus 1]	Cladosporium cladosporioides virus 1	*Polymycovirus*	266	266	68%	3.00E-73	32.26	760	YP_009052470.1
	RdRp [Plasmopara viticola lesion associated Polymycovirusmyco 1]	Plasmopara viticola lesion associated Polymycovirusmyco 1	*Polymycovirus*	265	265	68%	1.00E-72	31.5	763	QHG11067.1
	RNA-dependent RNA polymerase [Aspergillus spelaeus tetramycovirus 1]	Aspergillus spelaeus tetramycovirus 1	*Polymycovirus*	258	258	68%	3.00E-70	31.3	760	AYP71805.1
	RNA-dependent RNA polymerase [Phaeoacremonium minimum tetramycovirus 1]	Phaeoacremonium minimum tetramycovirus 1	Unclassified	257	257	68%	6.00E-70	32.09	764	QDB74985.1
	RdRp [Plasmopara viticola lesion associated Polymycovirusmyco 5]	Plasmopara viticola lesion associated Polymycovirusmyco 5	Unclassified	246	246	86%	3.00E-66	30.79	761	QHG11074.1
	RNA-dependent RNA-polymerase [Fusarium redolens polymycovirus 1]	Fusarium redolens polymycovirus 1	*Polymycovirus*	245	245	69%	9.00E-66	30.92	771	QDH44656.1
	RNA dependent RNA polymerase [Beauveria bassiana polymycovirus 2]	Beauveria bassiana polymycovirus 2	Unclassified	244	244	68%	2.00E-65	31.99	767	CUS18599.1
	RNA-dependent RNA polymerase [Penicillium digitatum polymycoviruses 1]	Penicillium digitatum polymycoviruses 1	*Polymycovirus*	243	243	75%	4.00E-65	27.67	760	YP_009551548.1
	RNA-dependent RNA polymerase [Colletotrichum camelliae filamentous virus 1]	Colletotrichum camelliae filamentous virus 1	*Polymycovirus*	239	239	68%	2.00E-63	31.03	771	ASV63092.1
	putative RNA-dependent-RNA-polymerase [Penicillium janthinellum polymycovirus 1]	Penicillium janthinellum polymycovirus 1	*Polymycovirus*, unassigned isolate	238	238	82%	3.00E-63	28.19	760	BCJ03666.1
	RNA-dependent RNA polymerase [Sclerotinia sclerotiorum tetramycovirus-1]	Sclerotinia sclerotiorum tetramycovirus-1	Unclassified	233	233	68%	5.00E-61	30.43	800	AWY10945.1
	RdRp [Uromyces virus B]	Uromyces virus B	Unclassified	230	230	69%	3.00E-60	26.97	772	QED43024.1

	RdRp [Plasmopara viticola lesion associated Polymycovirusmyco 3]	Plasmopara viticola lesion associated Polymycovirusmyco 3	Unclassified	226	226	70%	6.00E-59	30.58	763	QHG11072.1
	RNA-dependent RNA-polymerase [Alternaria tenuissima virus]	Alternaria tenuissima virus	Unclassified	174	174	44%	2.00E-42	31.41	537	AJP08049.1
	RdRp [Plasmopara viticola lesion associated Polymycovirusmyco 2]	Plasmopara viticola lesion associated Polymycovirusmyco 2	Unclassified	164	164	42%	1.00E-40	30.68	363	QHG11070.1
	RNA-dependent RNA polymerase [Beauveria bassiana polymycovirus 1]	Beauveria bassiana polymycovirus 1	*Polymycovirus*, unassigned isolate	158	158	33%	4.00E-39	36.82	292	VCV25415.1
	RNA-dependent RNA polymerase [Beauveria bassiana polymycovirus 1]	Beauveria bassiana polymycovirus 1	*Polymycovirus*, unassigned isolate	155	155	33%	3.00E-38	36.49	292	VCV25420.1
	RNA-dependent RNA polymerase [Beauveria bassiana polymycovirus 1]	Beauveria bassiana polymycovirus 1	*Polymycovirus*, unassigned isolate	155	155	33%	3.00E-38	36.49	292	VCV25416.1
	RNA-dependent RNA polymerase [Beauveria bassiana polymycovirus 1]	Beauveria bassiana polymycovirus 1	*Polymycovirus*, unassigned isolate	155	155	33%	6.00E-38	37.16	292	VCV25414.1
	RNA-dependent RNA polymerase [Beauveria bassiana polymycovirus 1]	Beauveria bassiana polymycovirus 1	*Polymycovirus*, unassigned isolate	154	154	33%	1.00E-37	36.15	292	VCV25413.1
	RNA-dependent RNA polymerase [Beauveria bassiana polymycovirus 1]	Beauveria bassiana polymycovirus 1	*Polymycovirus*, unassigned isolate	153	153	33%	2.00E-37	36.49	292	VCV25419.1
	RdRp [Plasmopara viticola lesion associated Polymycovirusmyco 4]	Plasmopara viticola lesion associated Polymycovirusmyco 4	Unclassified	115	115	29%	5.00E-24	33.46	307	QHG11073.1
	RNA dependent RNA polymerase [Mycovirus M7]	Mycovirus M7	Unclassified	65.5	65.5	12%	1.00E-08	33.94	116	QLF97276.1
	RNA dependent RNA polymerase [Beauveria bassiana polymycovirus 3]	Beauveria bassiana polymycovirus 3	Unclassified	47	47	6%	0.006	38.89	54	CUS18606.1
HadV1-1NL-RNA2	hypothetical protein [Hadaka virus 1]	Hadaka virus 1	Unclassified (Hadakavirus)	1343	1343	92%	0	91.67	720	BBU94039.1
	hypothetical protein [Colletotrichum camelliae filamentous virus 1]	Colletotrichum camelliae filamentous virus 1	*Polymycovirus*	58.9	58.9	42%	9.00E-05	24.58	692	ASV63093.1
HadV1-1NL-RNA3	methyltransferase [Hadaka virus 1]	Hadaka virus 1	Unclassified (Hadakavirus)	1229	1229	89%	0	92.32	671	BBU94040.1
	putative methyltransferase [Phakopsora virus B]	Phakopsora virus B	Unclassified	129	129	64%	2.00E-27	27.71	497	QED42895.1
	methyl transferase [Plasmopara viticola lesion associated Polymycovirusmyco 2]	Plasmopara viticola lesion associated Polymycovirusmyco 2	Unclassified	120	120	66%	3.00E-24	25.93	617	QHG11069.1
	putative methyltransferase [Penicillium janthinellum polymycovirus 1]	Penicillium janthinellum polymycovirus 1	*Polymycovirus*, unassigned isolate	118	118	72%	1.00E-23	25.68	612	BCJ03668.1
	unnamed protein product [Melampsora lini]	Melampsora lini	Unclassified	108	108	60%	2.00E-20	24.35	614	CAA45724.1
	methyltransferase [Penicillium digitatum polymycoviruses 1]	Penicillium digitatum polymycoviruses 1	*Polymycovirus*, unassigned isolate	107	107	61%	3.00E-20	26.39	612	YP_009551549.1
	hypothetical protein [Magnaporthe oryzae polymycovirus 1]	Magnaporthe oryzae polymycovirus 1	*Polymycovirus*	104	104	60%	3.00E-19	26.08	612	QAU09251.1
	methyl transferase [Beauveria bassiana polymycovirus 1]	Beauveria bassiana polymycovirus 1	*Polymycovirus*	95.9	95.9	66%	2.00E-16	25	610	YP_009352877.1

	Methyl transferase [Aspergillus fumigatus tetramycovirus-1]	Aspergillus fumigatus tetramycovirus-1	*Polymycovirus*	93.2	93.2	66%	1.00E-15	24.38	614	CDP74620.1
	methyltransferase [Penicillium brevicompactum tetramycovirus 1]	Penicillium brevicompactum tetramycovirus 1	*Polymycovirus*	92	92	63%	3.00E-15	24.69	612	AYP71803.1
	methyl transferase [Aspergillus fumigatus polymycovirus 1]	Aspergillus fumigatus polymycovirus 1	*Polymycovirus*, unassigned isolate	89.7	89.7	62%	2.00E-14	24.69	622	YP_009551546.1
	putative methyltransferase [Colletotrichum camelliae filamentous virus 1]	Colletotrichum camelliae filamentous virus 1	*Polymycovirus*	70.1	70.1	48%	3.00E-08	27.54	618	ASV63094.1
	methyltransferase [Aspergillus spelaeus tetramycovirus 1]	Aspergillus spelaeus tetramycovirus 1	*Polymycovirus*	64.3	64.3	46%	1.00E-06	25.41	448	AYP71807.1
HadV1-1NL-RNA4	hypothetical protein [Hadaka virus 1]	Hadaka virus 1	Unclassified (Hadakavirus)	422	422	54%	6.00E-144	81.03	255	BBU94041.1
HadV1-1NL-RNA5	hypothetical protein [Hadaka virus 1]	Hadaka virus 1	Unclassified (Hadakavirus)	409	409	60%	2.00E-140	84.91	233	BBU94042.1
HadV1-1NL-RNA6	hypothetical protein [Hadaka virus 1]	Hadaka virus 1	Unclassified (Hadakavirus)	399	399	66%	6.00E-137	85.02	257	BBU94044.1
HadV1-1NL-RNA7	hypothetical protein [Hadaka virus 1]	Hadaka virus 1	Unclassified (Hadakavirus)	107	107	31%	9.00E-25	50.45	111	BBU94043.1
HadV1-1NL-RNA8	hypothetical protein [Hadaka virus 1]	Hadaka virus 1	Unclassified (Hadakavirus)	134	134	50%	1.00E-34	49.38	161	BBU94046.1
HadV1-1NL-RNA9	hypothetical protein [Hadaka virus 1]	Hadaka virus 1	Unclassified (Hadakavirus)	207	207	46%	3.00E-63	68.97	148	BBU94047.1
HadV1-1NL-RNA10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

References

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