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ANNOTATED SEQUENCE RECORD

A novel narnavirus isolated from the wheat stripe rust fungus *Puccinia striiformis* f. sp. *tritici*

Yanhui Zhang[1] · Jing Zhao[1] · Xiaofei Liang[1] · Li Zheng[1,2] · Zhensheng Kang[1]

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Abstract

The complete genome of a novel fungal virus, Puccinia striiformis narnavirus 1 (PsNV1), was sequenced and analyzed. The full-length cDNA sequence is 2340 bp in length with a GC content of 50.04%. PsNV1 contains a single open reading frame (ORF), which encodes a putative RNA-dependent RNA polymerase (RdRp) of 741 amino acids with a molecular mass of 81.8 kDa. RdRp phylogeny showed that PsNV1 grouped together with Fusarium poae narnavirus 1 (FpNV1) as a sister branch of narnaviruses, forming a distinct clade. The results of genome sequence comparisons and phylogenetic analysis indicate that PsNV1 is a new member in the genus *Narnavirus*. To our knowledge, this is the first report of a narnavirus genome sequence in the obligately parasitic fungus *Puccinia striiformis* f. sp. *tritici*.

Wheat stripe rust (yellow rust), caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important diseases of wheat (*Triticum aestivum*) worldwide [1]. *P. striiformis* is an obligate biotrophic parasite that cannot be cultured on artificial medium and requires both primary (wheat or grasses) and alternate (*Berberis or Mahonia* spp.) host plants to complete its life cycle [2–4]. Mycoviruses have been commonly associated with all major lineages of plant-pathogenic fungi [5]. Rusts were first confirmed to harbor dsRNA mycoviruses in 1985 [6]. So far, however, only five mycovirus genomes parasitizing *P. striiformis* have been reported [7, 8]. The reasons for the shortage of research data on mycoviruses in *P. striiformis* are that it is difficult to obtain biological material and that many mycoviruses cause asymptomatic infections.

The family *Narnaviridae* currently comprises two genera, *Narnavirus* and *Mitovirus*. The genome of viruses of the family *Narnaviridae* is composed of a positive-sense single-stranded RNA segment (2.2 to 5.0 kb) containing a single open reading frame (ORF) encoding an RNA-dependent RNA polymerase (RdRp) [9–11]. A novel mitovirus in *P. striiformis* was first reported in 2019 in our laboratory [8]. In the present study, we report another novel mycovirus, Puccinia striiformis narnavirus 1 (PsNV1), infecting *P. striiformis* strain SCSN-12. Genome sequence alignment and phylogenetic analysis indicate that PsNV1 is a new member of the genus *Narnavirus* belonging to the family *Narnaviridae*.

Provenance of the virus material

P. striiformis strain SCSN-12 was isolated from a urediniospore pustule infecting wheat in Sichuan province, China, in 2015. This *P. striiformis* strain was propagated on the susceptible wheat variety Mingxian 169 as described previously [8], and the urediospores were harvested and stored at 4 °C until further use [12]. dsRNA was obtained from 0.5 g of urediniospores by selective absorption to a cellulose powder CF-11 column containing 16% ethanol [8]. The extracted dsRNAs were further treated with DNase I and S1 nuclease (Takara) to remove genomic DNA and ssRNA as described previously (Fig. 1A) [7]. A cDNA library of the purified dsRNA sample was constructed using an NEBNext®Ultra™

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✉ Li Zheng
zhenglihappy0617@126.com

✉ Zhensheng Kang
kangzs@nwsuaf.edu.cn

[1] State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi, China

[2] Key Laboratory of Green Prevention and Control of Tropical Plant Disease and Pests, Ministry of Education and College of Plant Protection, Hainan University, Haikou 570228, Hainan, China

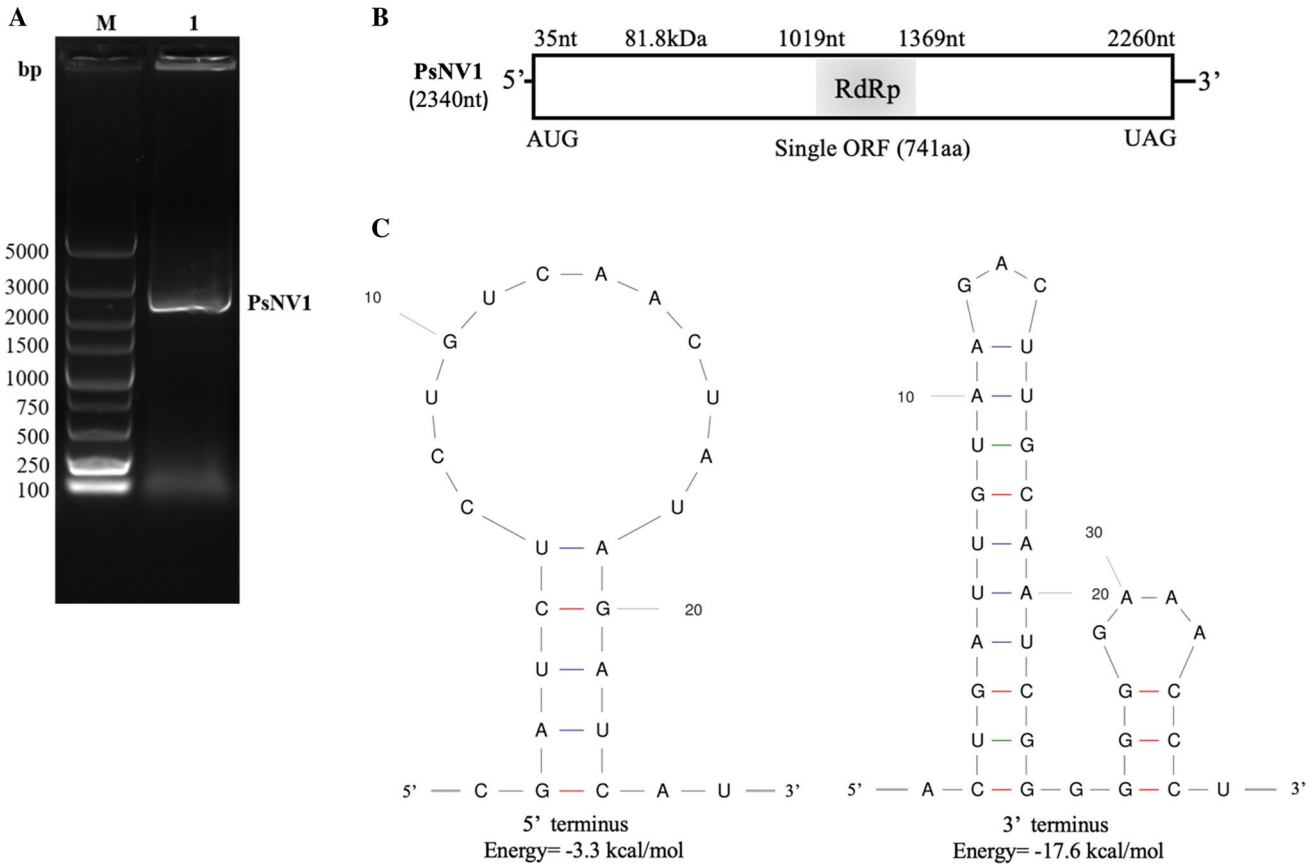

Fig. 1 (**A**) Agarose gel electrophoresis of dsRNA extracted from urediniospores of *P. striiformis* treated with DNase I and S1 nuclease. M indicates molecular markers of DNA Ladder 5000. (**B**) Schematic representation of the genomic organization of PsNV1. The open reading frame (ORF) and the untranslated regions (UTRs) are indicates by an open bar and single lines, respectively. The red bar indicated the conserved RdRp domain. The numbers above the ORF indicate the nucleotide positions of the initiation and termination codons, respectively. The molecular weight of the predicted protein is shown above the ORF. The initiation and termination codons are shown below the ORFs. (**C**) Predicted secondary structures of the 5' (left) and 3'termini (right) of PsNV1

RNA Library Prep Kit (Illumina) according to the manufacturer's protocol. To obtain the terminal sequences of the dsRNA, the 5' and 3' termini were amplified by rapid amplification of cDNA ends (RACE kit, Invitrogen) [13]. The amplified PCR product was cloned and sequenced by the Sanger method. The sequence was assembled using SeqMan from the Lasergene sequence analysis package (DNASTAR). The complete nucleotide sequence of PsNV1 has been submitted to the GenBank database under the accession number MN756800. The putative ORF was identified using the ORF Finder program (http://www.ncbi.nlm.gov/ gorf/gorf.html). A protein domain search was performed using the Conserved Domain Database (CDD) (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Multiple sequence alignment of the protein sequences were performed with ClustalW 2.0 [14] and DNAMAN 7.0 software (Lynnon Biosoft, USA). A phylogenetic tree was generated by the maximum-likelihood (ML) method in MEGA 7.0 software with 1000 bootstrap replicates [15].

Sequence properties

The full-length cDNA sequence of the PsNV1 genome is 2,340 nt in length with a GC content of 50.04%. The 5′ and 3′ untranslated regions (UTRs) of PSNV1 are 34 and 80 nt in length, respectively (Fig. 1B). Using the Unafold program (http://unafold.rna.albany.edu), two potential stem-loop structures were predicted, and the initial ΔG values of 5′ and 3′ UTRs were -20.30 kcal/mol and -40.20 kcal/mol, respectively (Fig. 1C). Using the standard genetic code, the PsNV1 genome contained a single ORF encoding a 741-amino-acid (aa) protein with a molecular mass of 81.8 kDa (Fig. 1B).

A sequence search using the BLASTp tool showed that this 81.8-kDa protein had significant sequence similarity to the RNA-dependent RNA polymerases (RdRps) of narnaviruses. Fusarium poae narnavirus 1 (FpNV1) [16] was the best match (29.6% identity), followed by

Saccharomyces 23S RNA narnavirus (26.96% identity) and Saccharomyces 20S RNA narnavirus (24.9% identity) [9]. Furthermore, a CDD search and multiple protein sequence alignments indicated that the RdRp domain includes six conserved motifs (Fig. 2A) that are characteristic of members of the genus *Narnavirus*. These results suggest that *P. striiformis* strain SCSN-12 was infected by a novel narnavirus, PsNV1.

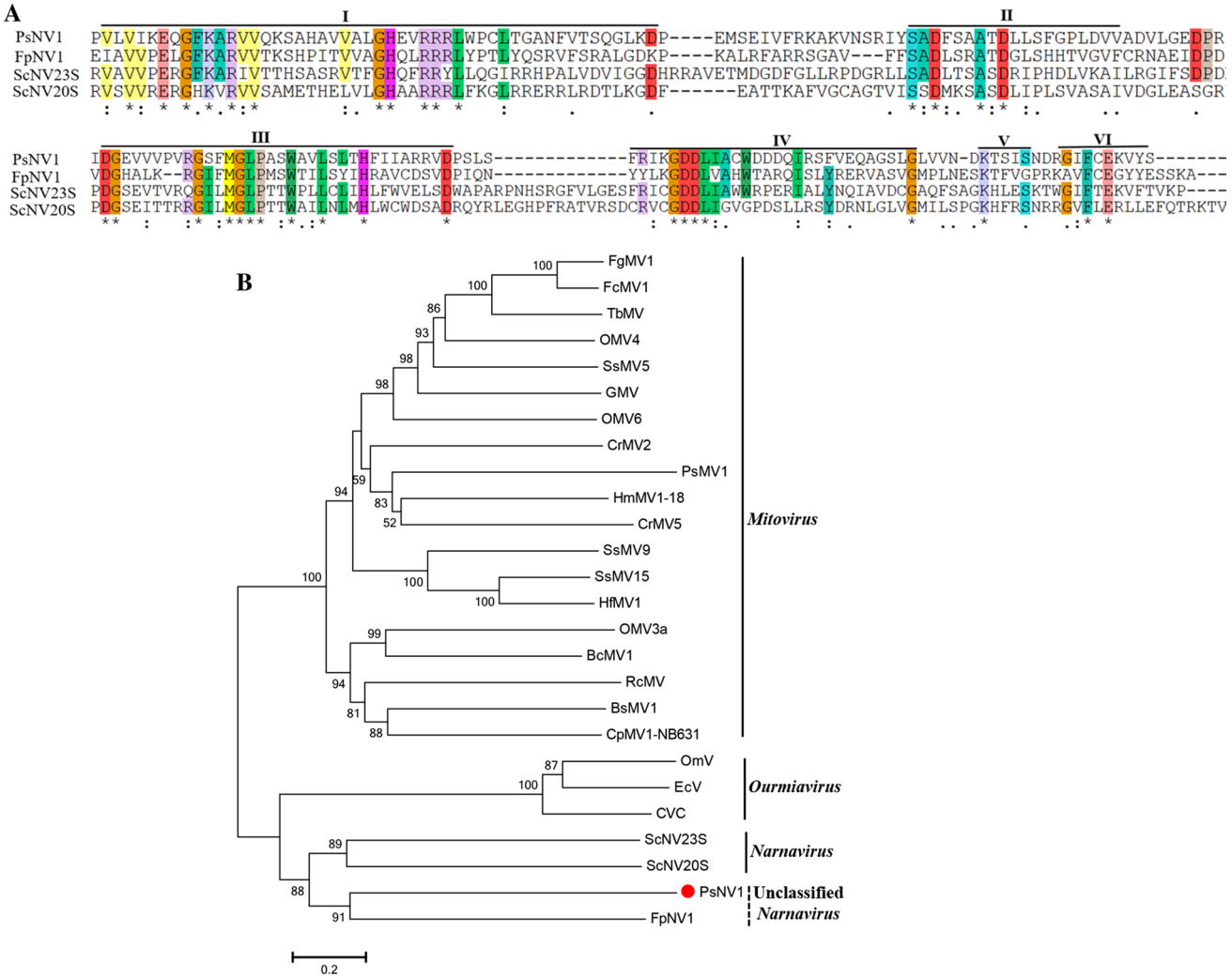

Fig. 2 (**A**) Sequence alignment of the RdRp amino acid sequences of PsNV1 and representative members of the genus *Narnavirus*. Horizontal lines above the alignment indicate the six conserved motifs. Shaded areas indicate identical amino acid residues. Asterisks, colons, and dots indicate identical amino acid residues, conservative variations, and semi-conservative variations, respectively. (**B**) Phylogenetic tree generated by the maximum-likelihood (ML) method from the alignment of the RdRp amino acid sequences of PsNV1 and members of the family *Narnaviridae*, using MEGA (version 7.0). The numbers above the internal branches indicate bootstrap values estimated based on 1,000 replications. The scale bar represents the estimated number of amino acid substitutions per site. The abbreviation of virus names and GenBank accession numbers are as follows: FgMV1, Fusarium globosum mitovirus 1 (YP_009126872.1); FcMV1, Fusarium circinatum mitovirus 1 (AHI43533.1); TbMV, Thielaviopsis basicola mitovirus (YP_00282222 9.1); OMV4, Ophiostoma mitovirus 4 (NP_660179.1); SsMV5, Sclerotinia sclerotiorum mitovirus 5 (AHX84132.1); GMV, Gremmeniella mitovirus (CCD32685.2); OMV6, Ophiostoma mitovirus 6 (NP_660181.1); SsMV15, Sclerotinia sclerotiorum mitovirus 15 (AHF48631.1); HfMV1, Hymenoscyphus fraxineus mitovirus 1 (AIU44705. 1); SsMV9, Sclerotinia sclerotiorum mitovirus 9 (AHF48625.1); CrMV2, Cronartium ribicola mitovirus 2 (AMQ 67415.1); CrMV5, Cronartium ribicola mitovirus 5 (YP_009259487.1); PsMV1, Puccinia striiformis mitovirus 1 (MK033478); HmMV1-18, Helicobasidium mompa mitovirus 1-18 (BAD72871.1); OMV3a, Ophiostoma mitovirus 3a (NP_660176.1); BcMV1, Botrytis cinerea mitovirus 1 (CEZ26300.1); RcMV, Rhizoctonia cerealis mitovirus (AIT71973.1); BsMV1, Buergenerula spartinae mitovirus 1 (AHY03257.1); CpMV1-NB631, Cryphonectria parasitica mitovirus 1-NB631 (NP_660174.1); OMV, Ourmia melon virus (ACF16360.1); ECV, Epirus cherry virus (ACF16357.1); CVC, cassava virus C (ACI03053.1); ScNV23S, Saccharomyces 23S RNA narnavirus (NP_660177.1); ScNV20S, Saccharomyces 20S RNA narnavirus (NP_66017 8.1); FpNV1, Fusarium poae narnavirus 1(YP_009272902.1)

To investigate the relationship between PsNV1 and other mycoviruses, a molecular phylogenetic tree was constructed based on the RdRp amino acid sequences of PsNV1, other narnaviruses, mitoviruses, and plant ourmiaviruses. The phylogenetic tree revealed that PsNV1 grouped together with FpNV1 as a sister branch of narnaviruses, forming a distinct clade. Therefore, the phylogenetic tree clearly placed PsNV1 in a separate cluster containing FpNV1 and unclassified narnaviruses (Fig. 2B). To our knowledge, this is the first report of the full-length nucleotide sequence of a novel narnavirus in the obligate parasitic fungus *P. striiformis.*

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

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

References

1. Wellings CR (2011) Global status of stripe rust: a review of historical and current threats. Euphytica 179:129–141
2. Zheng WM, Huang LL, Huang JQ, Wang XJ, Chen XM, Zhao J (eds) (2014) High genome heterozygosity and endemic genetic recombination in the wheat stripe rust fungus. Nat. Commun 5: 3134–3134
3. Milus EA, Kristensen K, Hovmøller MS (2009) Evidence for increased aggressiveness in a recent widespread strain of *Puccinia striiformis* f. sp. *tritici* causing stripe rust of wheat. Phytopathology 99:89–94
4. Hovmøller MS, Walter S, Justesen AF (2010) Escalating threat of wheat rusts. Science 329:369
5. Ghabrial SA, Jiang D, Nibert ML, Suzuki N (2015) 50-plus years of fungal viruses. Virology 479:356–368
6. Newton AC, Caten CE, Johnson R (1985) Variation for isozymes and double-stranded RNA among isolates of *Puccinia striiformis* and two other cereal rusts. Plant Pathol 34:235–247
7. Zheng L, Lu X, Liang XF, Jiang SC, Zhao J, Zhan GM, Liu P, Wu JH, Kang ZS (2017) Molecular characterization of novel totivirus-like double-stranded RNA from *Puccinia striiformis* f. sp. *tritici*, the causal agent of wheat stripe rust. Front Microbiol 8:1960
8. Zheng L, Zhao J, Liang X, Zhuang H, Qi T, Kang Z (2019) Complete genome sequence of a novel mitovirus from the wheat stripe rust fungus *Puccinia striiformis*. Arch Virol 164:897–901
9. Hillman BI, Cai G (2013) The family *Narnaviridae*: simplest of RNA viruses. Adv Virus Res 86:149–176
10. Wickner RB, Fujimura T, Esteban R (2013) Viruses and prions of *Saccharomyces cerevisiae*. Adv Virus Res 86:1–36
11. Vainio EJ (2019) Mitoviruses in the conifer root rot pathogens *Heterobasidion annosum* and *H. parviporum*. Virus Res 271:1–5
12. Cao LH, Xu SC, Chen WQ, Liu TG, Chen WQ (2008) Early molecular diagnosis and detection of *Puccinia striiformis* f. sp. *tritici* in China. Lett Appl Microbiol 46:501–506
13. Komatsu K, Katayama Y, Omatsu T, Mizutani T, Fukuhara T, Kodama M, Arie T, Teraoka T, Moriyama H (2016) Genome sequence of a novel mitovirus identified in the phytopathogenic fungus *Alternaria arborescens*. Arch Virol 161:2627–2631
14. Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG (2003) Multiple sequence alignment with the clustal series of programs. Nucleic Acids Res 31:3497–3500
15. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
16. Osaki H, Sasaki A, Nomiyama K, Tomioka K (2016) Multiple virus infection in a single strain of *Fusarium poae* shown by deep sequencing. Virus Genes 52:835–847