Archives of Virology (2024) 169:128
https://doi.org/10.1007/s00705-024-06054-y

ANNOTATED SEQUENCE RECORD

Molecular characterization of a single-negative-stranded RNA virus from the rice blast fungus *Magnaporthe oryzae* isolate NJ39

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Received: 23 December 2023 / Accepted: 29 February 2024 / Published online: 28 May 2024

Abstract

A novel negative-sense single-stranded RNA mycovirus, designated as "Magnaporthe oryzae mymonavirus 1" (MoMNV1), was identified in the rice blast fungus *Magnaporthe oryzae* isolate NJ39. MoMNV1 has a single genomic RNA segment consisting of 10,515 nucleotides, which contains six open reading frames. The largest open reading frame contains 5837 bases and encodes an RNA replicase. The six open reading frames have no overlap and are arranged linearly on the genome, but the spacing of the genes is small, with a maximum of 315 bases and a minimum of 80 bases. Genome comparison and phylogenetic analysis indicated that MoMNV1 is a new member of the genus *Penicillimonavirus* of the family *Mymonaviridae*.

Mycoviruses, or fungal viruses, are extremely diverse and can have either RNA or DNA genomes. They are widespread in all major groups of plant-pathogenic fungi, including Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota, and Neocallimastigomycota [1]. Although only a few mycoviruses are known to have adverse effects on their fungal hosts, such as decreased virulence, irregular growth, abnormal pigmentation, or defects in sexual development, these examples highlight the potential value of yet undiscovered mycoviruses as disease control agents in agriculture and forestry [2–4].

Only a few known mycoviruses have genomes consisting of linear negative-sense single-stranded RNA ((–) ssRNA) or circular single-stranded DNA (ssDNA) [5, 6], and most mycovirus genomes are double-stranded RNA (dsRNA) or linear positive-sense single-stranded RNA ((+) ssRNA) [7]. The *Mymonaviridae* are a family of linear negative-sense single-stranded RNA ((–) ssRNA) viruses that are most closely related to members of the families *Bornaviridae*, *Lispiviridae*, *Nyamiviridae*, and *Rhabdoviridae* (ICTV Virus Taxonomy, 2023 Release https://talk.ictvonline.org/taxonomy/). The family *Mymonaviridae* comprises nine genera: *Auricularimonavirus*, *Botrytimonavirus*, *Hubramonavirus*, *Lentimonavirus*, *Penicillimonavirus*, *Phyllomonavirus*, *Plasmopamonavirus*, *Rhizomonavirus*, and *Sclerotimonavirus*. One member of the family *Mymonaviridae*, Sclerotinia sclerotiorum negative-stranded RNA virus 1 (SsNSRV-1), produces filamentous, enveloped virions containing a single molecule of linear, negative-sense RNA of about 10 kb [8], but whether other members of this family also produce virions is unknown. Mymonavirids are mainly hosted by filamentous fungi and have been identified in metagenomic studies of plant-pathogenic, edible, and endophytic fungi [8–10].

Magnaporthe oryzae (teleomorph) (Herbert) Barr (anamorph: Pyricularia oryzae) is a filamentous fungus that causes rice blast. This disease is extremely destructive to rice and causes significant yield losses globally every year [11–14]. Several mycoviruses have been identified in *M. oryzae*, including dsRNA viruses and (+) ssRNA viruses [15]. The dsRNA viruses include Magnaporthe oryzae chrysovirus 1-A (MoCV1-A) of the family *Chrysoviridae,* Magnaporthe oryzae partitivirus 1 (MoPV1) of the family *Partitiviridae*, and Magnaporthe oryzae virus 1 (MoV1) of the family *Pseudototiviridae* [16–18]. The (+) ssRNA viruses include Magnaporthe oryzae narnavirus 1 (MoNV1) of the family *Narnaviridae* and Magnaporthe oryzae virus A (MoVA) of the family *Tombusviridae* [11, 19]. In addition, the (+) ssRNA viruses Magnaporthe oryzae ourmia-like viruses 1 and 4 (MOLV1 and MOLV4), Pyricularia oryzae ourmia-like viruses 1, 2, and 3 (PoOLV1, PoOLV2, and PoOLV3), and Magnaporthe

Handling Editor: Massimo Turina

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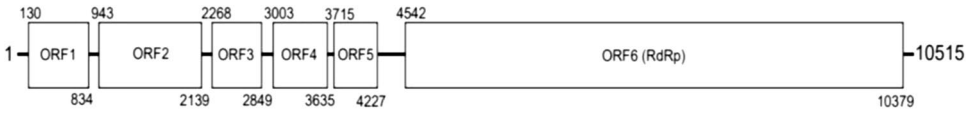

Fig. 1 MoMNV1 genome structure and characteristics. Rectangular boxes represent ORFs, with ORF6 encoding the RDRP. The starting and ending nucleotide position of each ORF is indicated

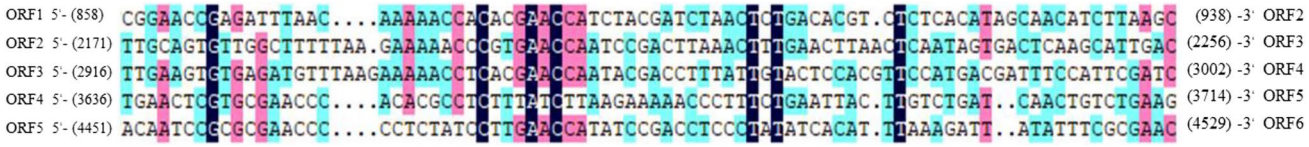

Fig. 2 Comparison of nucleotide bases in the five UTRs separating the six ORFs

oryzae botourmia-like viruses 5, 6, and 7 (MoBV5, MoBV6, and MoBV7), all of which belong to the family *Botourmiaviridae*, have also been identified in *M. oryzae* [7, 20, 21].

Virus isolation and sequencing

M. oryzae strain NJ39 was isolated from a lesion of a rice neck-panicle sample that was collected in the city of Nanjing, Jiangsu province, China, and it showed an abnormal biological phenotype with decreased pigmentation, fewer aerial hyphae, and no production of conidia. High-throughput RNA sequencing revealed that strain NJ39 was infected with a single novel virus, which was tentatively named "Magnaporthe oryzae mymonavirus 1" (MoMNV1).

M. oryzae strains were stored on rice stem nodes at -20 °C and cultured on potato dextrose agar at 28 °C. Specific primers based on the high-throughput sequencing results were used to determine the complete genome sequence and used for RT-PCR to confirm the accuracy of the original

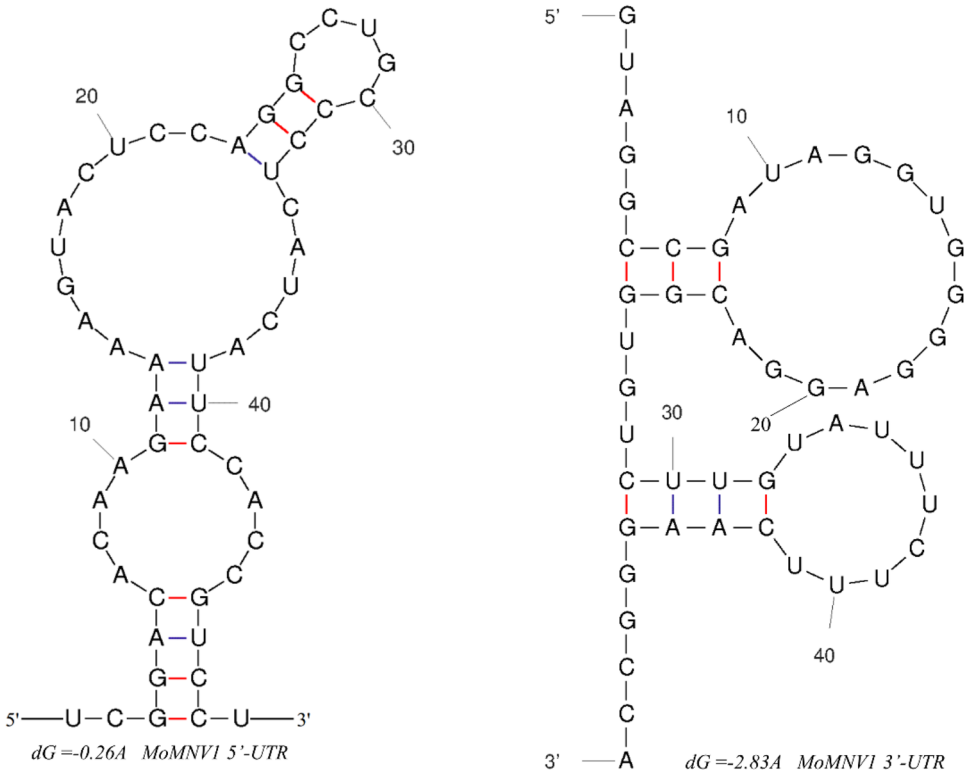

Fig. 3 Potential RNA secondary structures in the 5′- and 3′-terminal sequences of MoMNV1. Stem-loop structures were predicted in both termini, with ΔG values of -0.26 and -2.83 kcal/mol, respectively. Short lines in different colors indicate hydrogen bonds between different base pairs (red, G-C pairs; purple, A-U pairs)

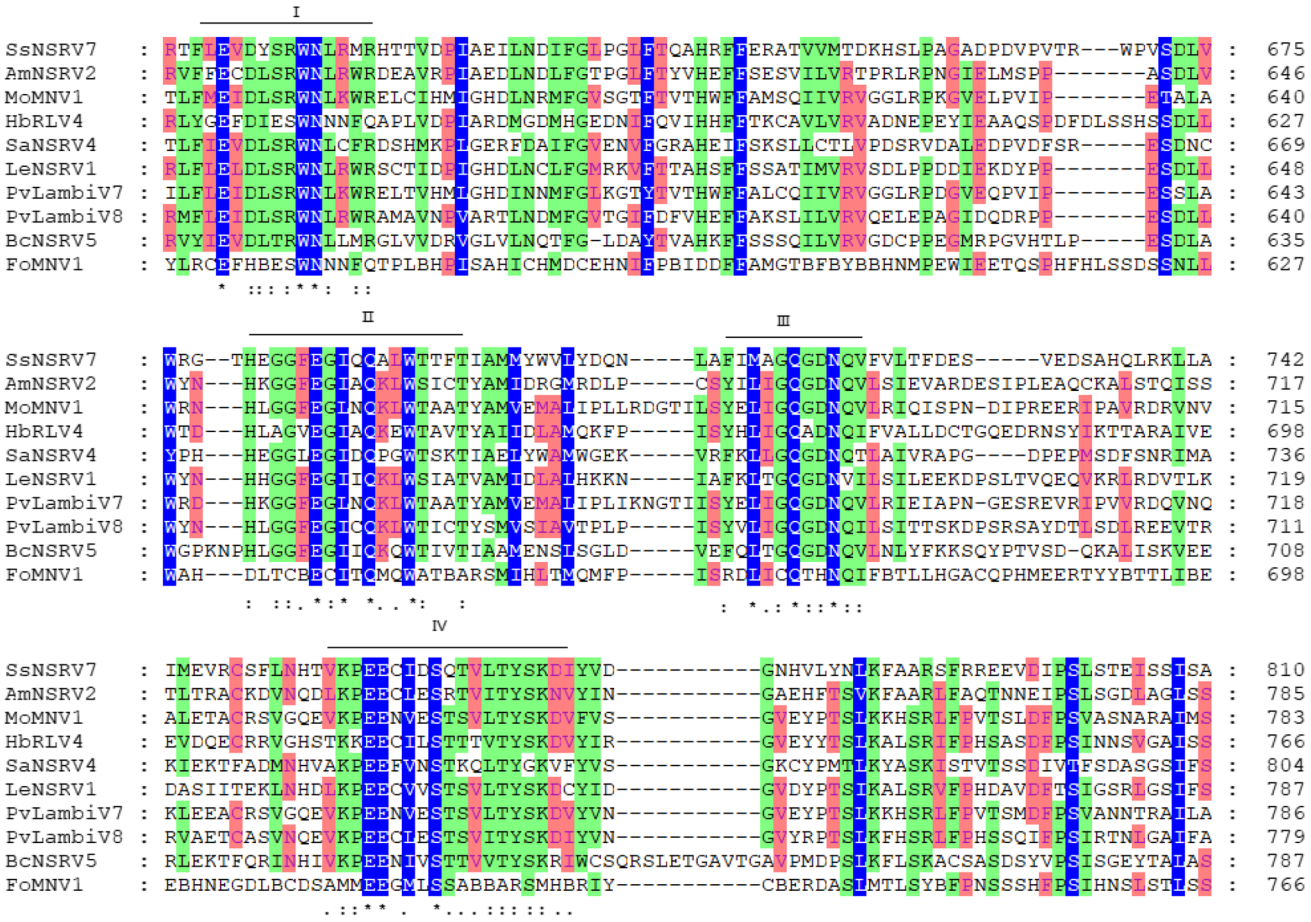

Fig. 4 Multiple alignment of the amino acid (aa) sequences of RDRPs encoded by MoMNV1 and other selected members of the family *Mymonaviridae*. The four conserved RDRP motifs are indicated by the Roman numerals I to IV. Asterisks represent identical amino acid residues, colons represent highly conserved residues, and single dots represent less conserved but related residues. Virus names are abbreviated as follows: SsNSRV7, Sclerotinia sclerotiorum negative-stranded RNA virus 7 (AWY11040.1); AmNSRV2, Armillaria mellea negative strand RNA virus 2 (BK014417.1); MoMNV1, Magnaporthe oryzae mononegaambi virus 1 (OL415836.1); HbRLV4, Hubei rhabdo-like virus 4 (YP_009336595.1); SaNSRV1, soybean leaf-associated negative-stranded RNA virus 4 (YP_010784559.1); LeNSRV1, Lentinula edodes negative-strand RNA virus 1 (YP_010796439.1); PvLambiV7, Plasmopara viticola lesion associated mononegaambi virus 7 (YP_010798439.1); PvLambiV8, Plasmopara viticola lesion associated mononegaambi virus 8 (YP_010798440.1); BcNSRV5, Botrytis cinerea negative-stranded RNA virus 5 (YP_010800334.1); FoMNV1, Fusarium oxysporum mymonavirus 1 (YP_010805694.1)

sequence by resequencing. A ligase-mediated method was used to amplify cDNA at the 5 'and 3' ends in order to determine the terminal sequences of the dsRNA [16, 19, 22]. Each base pair was determined in both orientations by sequencing three or more independent overlapping clones. The complete nucleotide sequence of the MoMNV1 genome has been deposited in the GenBank database with the accession number OL415836.1. The amino acid (aa) sequence of the putative RdRp of MoMNV1 was aligned with other virus RdRp sequences using the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/). RNA secondary structures in the terminal regions of the genome were predicted using the UNAFold web server (http://www.unafold.org/Dinamelt/applications/quickfold.php) [23]. Conserved domains were identified using the NCBI Conserved Domain Database (https://www.ncbi.nlm.nih.gov/cdd). Based on the aligned sequences, a phylogenetic tree was constructed by the neighbor-joining method using MEGA version 6.0 [24].

Sequence properties

The full-length nucleotide sequence of the MoMNV1 genome is 10,515 nucleotides (nt) long with a GC content of 48.2%. Using the standard genetic code, it was predicted to contain six open reading frames (ORFs; ORF1-ORF6), with ORF6 being the largest, consisting of 5837 nt, starting at nucleotide position 4542 and ending at position 10,379,

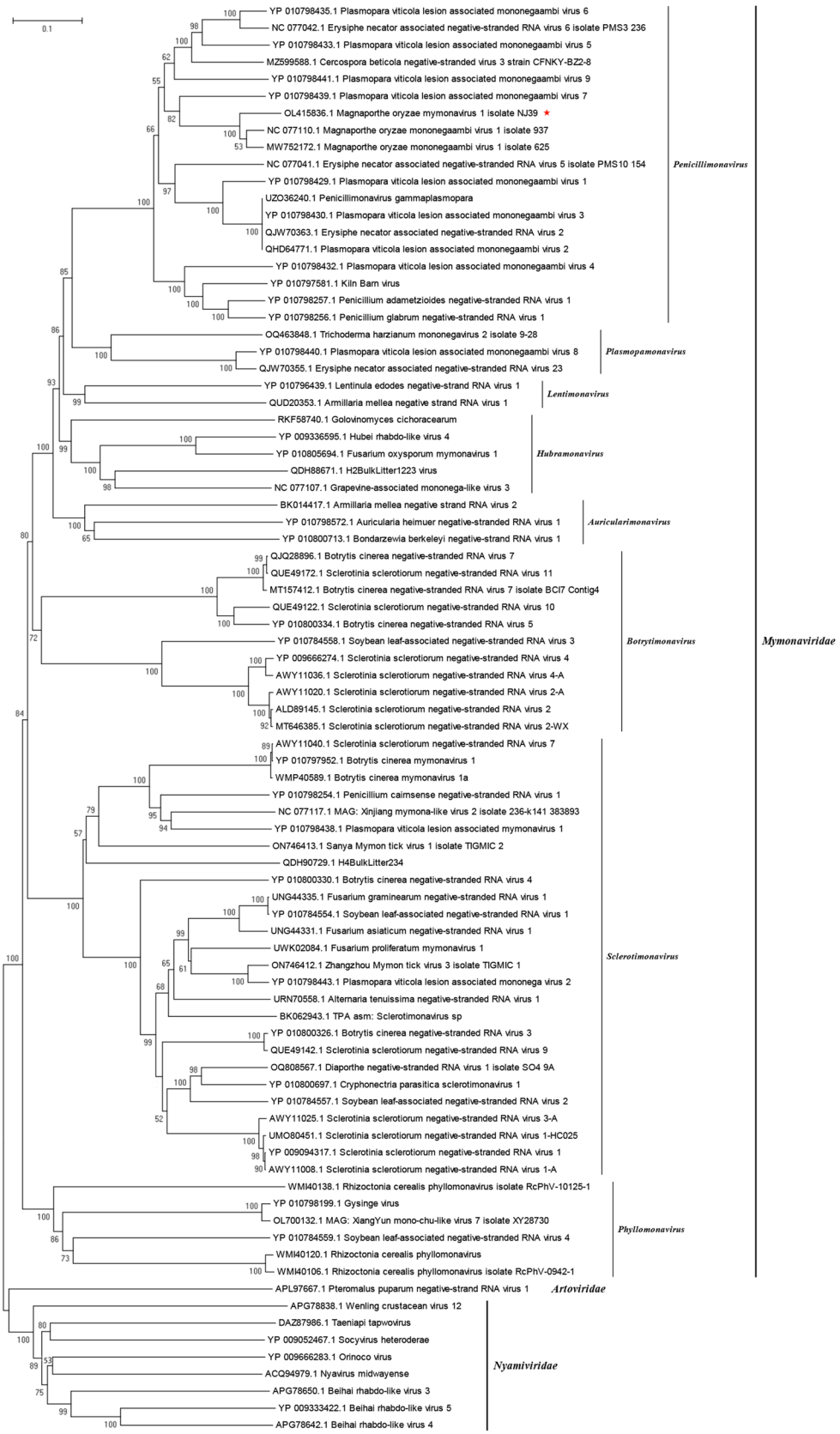

Fig. 5 Phylogenetic analysis based on the RDRP sequences of MoMNV1 and related viruses of the families *Mymonaviridae*, *Artoviridae*, and *Nyamiviridae*. The phylogenetic tree was constructed by the neighbor-joining method using the program MEGA 6.0. Bootstrap values (1000 replicates) are shown at the nodes, and the scale bar (0.1) corresponds to the genetic distance. The RdRp sequences were obtained from the GenBank database, and the accession numbers are shown before the taxon names. The position of MoMNV1 is indicated by a red star. The tree was rooted with four viruses of the family *Fiersviridae* (formerly *Leviviridae*). Only *p*-values of the approximate likelihood ratios (SH-test) >0.5 (50%) are indicated. The scale bar corresponds to 0.5 amino acid substitutions per site

and encoding an RNA-dependent RNA polymerase (RDRP). ORF5 is the smallest ORF, consisting of 512 nt, starting at nucleotide position 3715 and ending at position 4227. The MoMNV1 genome has a 5' untranslated region (UTR) of 130 nt and a 3'-UTR of 136 nt, and no significant complementarity between the UTRs was found (Fig. 1).

ORFs 1-5 encode proteins of 704, 1196, 581, 632, and 512 aa, respectively. The nucleic acid base consistency rate of the five UTRs separating the six ORFs was found to be 58.6% (Fig 2). The 5'-terminal sequence (nt positions 1-60) and the 3'-terminal sequence (nt positions 10,470-10,515) of MoMNV1 were predicted to be folded into potentially stable stem-loop structures with ΔG values of -3.03 and -2.11 kcal/mol, respectively (Fig. 3).

A homology search of the GenBank database using BLASTp showed that the MoMNV1 RdRp was most closely related to the RdRps of some members of the genus *Penicillimonavirus* in the family *Mymonaviridae*, including Plasmopara viticola lesion associated mononegaambi virus 7 (YP_010798439.1; identity, 59%; query coverage, 99%; e-value, 0), Magnaporthe oryzae mononegaambi virus 1 (NC_077110.1; identity, 90%; query coverage, 99%; e-value, 0), and Erysiphe necator associated negative-stranded RNA virus 6 (NC_077042.1; identity, 52%; query coverage 99%; e-value, 0). Furthermore, a conserved domain database search and a multiple amino acid sequence alignment confirmed that the protein encoded by MoMNV1 contains four typical conserved motifs that are characteristic of the RdRps of (-) ssRNA viruses [25] (Fig. 4). To determine the taxonomic position of MoMNV1, a molecular phylogenetic tree was constructed using aa sequences of the RdRp regions of MoMNV1 and 83 other selected viruses of the families *Mymonaviridae*, *Artoviridae*, and *Nyamiviridae*. Nine viruses of the families *Artoviridae* and *Nyamiviridae* were used as an outgroup. As shown in Fig. 4, the neighbor-joining tree strongly suggested that MoMNV1 is a new member of the family *Mymonaviridae* (Fig. 5).

Funding This research was supported by the National Natural Science Foundation of China (NFSC No. 31201474).

Data Availability The authors confirm that the data supporting the findings of this study are available within the article [and/or its supplementary materials].

Declarations

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

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

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