A Novel Fungal Negative-strand RNA Virus Related to Mymonaviruses in *Auricularia heimuer*

Yongping Fu (fuyongping@jlau.edu.cn)
Jilin Agricultural University https://orcid.org/0000-0003-4966-701X
Xufei Li
Jilin Agricultural University
Qingcheng Liu
Guizhou Agricultural Sciences: Guizhou Provincial Academy of Agricultural Sciences
Shiyu Li
Jilin Agricultural University
Frederick Leo Sossah
Jilin Agricultural University
Xuerong Han
Jilin Agricultural University
Guosheng Zhu
Guizhou Agricultural Sciences: Guizhou Provincial Academy of Agricultural Sciences
Yu Li
Jilin Agricultural University
Changtian Li
Jilin Agricultural University

Research Article

Keywords:

Posted Date: April 7th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1454350/v1

Abstract

We reported a novel (-)ssRNA mycovirus isolated from Auricularia heimuer CCMJ1222, using a combination of RNA-seq sequencing, reverse transcription-polymerase chain reaction, 5' and 3' rapid amplification of cDNA ends, and Sanger sequencing. Combining database searches, sequence alignment, and phylogenetic analysis, we designated the virus as Auricularia heimuer negative-stranded RNA virus 1 (AhNsRV1). The virus had a monopartite RNA genome related to mymonaviruses (Mononegavirales). The AhNsRV1 genome consists of 11,441 nucleotides and six linear open reading frames (ORFs). The largest ORF encodes a large protein; the second ORF encodes a hypothetical protein, while other ORF functions were unknown. AhNsRV1 was the first (-)ssRNA virus known to infect fungi and was the third virus to be discovered from *A. heimuer*.

Full Text

Fungal viruses exist widely in various fungal taxa, including yeasts, mushrooms, and pathogenic fungi that infect plants, insects, and humans [1-3]. In 1948, the first such virus was found in the edible fungus *Agaricus bisporus* [4]. Examples of viruses or virus particles in different mushroom species include Mushroom bacilliform virus (MBV), an ssRNA virus, and Mushroom virus X (MVX), a dsRNA virus, are found in *A. bisporus* [5-7]; *Lentinula edodes* spherical virus, a ssRNA virus, is found in *Lentinula edodes* [8], and Flammulina velutipes browning virus (FvBV), a dsRNA virus, is found in *Flammulina filiformis* [9,10].

Viral metagenomics is a new virus detection method based on the rapid development of high-throughput sequencing technology. The genomes of ssRNA and dsRNA viruses in edible mushrooms have been reported [11], but single negative-stranded RNA viruses are rarely reported. The single-strand negative-sense viruses officially announced as of 2018 comprise eight families: *Bornaviridae*, *Filoviridae*, *Mymonaviridae*, *Nyamiviridae*, *Paramyxoviridae*, *Pneumoviridae*, *Rhabdoviridae*, and *Sunviridae* [12]. The genome is a linear single segment RNA genome with a size of about 10 kb in the family *Mymonaviridae*, and the host range is mainly fungi [12,13]. The genome has no poly (A) tail structure at the 3' end and contains filamentous virus particles with a diameter of 25-50 nm and a length of about 1,000 nm [12,13]. In addition, the virus particles have an envelope structure [12,13].

Auricularia heimuer has been widely cultivated in China and adopted as a dietary supplement that possesses medicinal properties. However, the potential of reduced quality and yield relative to the virus has not been demonstrated yet. In this study, the collected strains of *A. heimuer* from local factories were subjected to RNA sequencing. Phylogenetic analysis showed that AhNsRV1 was a new member of the family *Mymonaviridae.* This is the first single negative-strand RNA virus and the third virus discovered from *A. heimuer*. Our research will enrich our knowledge of the negative RNA virus resources in fungi.

A. heimuer strain CCMJ1222 was collected from the Engineering Research Center of Edible and Medicinal Fungi, Ministry of Education, Jilin Agricultural University. The target strain was inoculated onto potato

dextrose agar (PDA; BD Difco Laboratories, Detroit, MI, USA) plates medium for seven days in the dark at 25°C.

Fungal total RNA was extracted using an RNAiso Kit according to the manufacturer's instructions (Takara, Dalian, China). The total RNA samples were treated with DNase I (Takara, Dalian, China) and evaluated for integrity by agarose gel electrophoresis and a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The RNA samples were depleted of rRNAs using an NEB Next rRNA Depletion Kit (New England Biolabs, Ipswich, MA, USA) and used as a template to construct paired-end libraries with a NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA). The cDNA library was sequenced (150-bp paired-end reads) on an Illumina NovaSeq 6000 (Illumina, USA). The library construction and deep sequencing were performed by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

The adapter sequences and low-quality reads were removed from the raw reads of each library using Trimmomatic (version 0.36) [14] after deep sequencing. The clean reads were mapped to the *A. heimuer* genome [15] using HISAT2 (version 2.1.0) [16]. The unmapped reads were assembled using Trinity (version 2.5.0) by de novo assembly [17](Haas et al., 2013). The assembled contigs were compared against the NCBI nonredundant protein database using BLASTn and BLASTX (with significant e-value ≤ 1e-5) to identify virus-related contigs. The virus-related contigs matched to the same reference sequence were merged into longer contigs using DNAMAN software (version 8.0; Lynnon Biosoft, Quebec, Canada).

Specific primers were designed based on the virus-related contigs and used for RT-PCR assays to identify viral sequences in the *A. heimuer* strains. First-strand cDNA was synthesized with a cDNA synthesis supermax kit (TransGen, Beijing, China) using the total RNAs of *A. heimuer* obtained previously as a template. The 18S rRNA gene was used as an internal reference gene to analyze the synthesized cDNA [18]. Based on the verified sequences, we identified a novel virus we named AhNsRV1-DW. The AhNsRV1-DW specific primers were used to locate the target strain by PCR (Table S1).

To verify the integrity of the virus genome, nested PCR was carried out with multiple sets of overlapping primers by the RACE method [18-21]. PCR products were separated by agarose gel electrophoresis, recovered using a Gel Extraction Kit (Axygen, NY, USA), and cloned into a pMD18-T vector (TaKaRa, Dalian, China). Primer M13-47/M13-48 (CGCCAGGGTTTTCCCAGTCACGAC/ AGCGGATAACAATTTCACACAGGA) and specific primers were used for PCR detection of cloned sequences in bacteria cultured in a liquid medium (Table S1). The liquid culture medium containing the target bacteria was sent to Wuhan Quintarabio Biotechnology Co., Ltd. for sequencing. Each sample was sequenced three times to obtain high-quality sequences and avoid false-positive PCR results. Finally, the full-length cDNA sequence of the virus in *A. heimuer* strain CCMJ1222 was obtained by using SnapGene splicing.

According to the whole genome sequence, 3–4 specific primers were designed by the NCBI online software primer blast to verify the accuracy of the full-length sequence, and the RACE method was used to verify the two-terminal sequence. Total RNA was extracted by RNAiso Kit (Takara, Dalian, China). The

KOD high fidelity enzyme was used for amplification. The PCR reaction system and conditions followed the instructions in the manual. At the same time, an Axygen recovery kit (Axygen, NY, USA) was used for gel cutting recovery, and a pTOPO-Blunt Kit (Aidlab, Beijing, China) was used for flat end cloning. The reaction system followed the kit instructions.

The conservative domain of AhNsRV1 was predicted by the online software motif (https://www.genome.jp/tools/motif/. The blast program of NCBI online software was used to search sequence homology (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was analyzed by the maximum likelihood method using PhyML 3.0 software [22,23]. Amino acid sequence alignment was performed using MAFFT and ClustalX software [24,25]. The phylogenetic tree was visualized and edited using MEGA-X.

The assembled genome of AhNsRV1 was 11,441 bp in length and had a GC content of 59.5%. The genome contained six ORFs with no overlaps (Fig.1A). There was no cap structure at the 5' end and no poly(A) structure at the 3' end. The virus contained 57-nt-short and 296-nt-long 5' and 3' UTRs, respectively. The 3'-UTR and 5'-UTR sequences had obvious complementary characteristics and were highly homologous (Fig.1B). The spacer region was a series of a highly homologous sequence rich in A / U (Fig.1C) and was highly conserved between the six ORFs. The largest ORF in AhNsRV1 is ORF □. ORF □ is 5,908 nt long and encodes a putative L protein of 1,969 amino acid residues with a calculated molecular weight of 220.59 kDa and an isoelectric point of 6.64. ORF □ putatively encodes a hypothetical protein. ORFs I, III, IV, and V had no homologous information in the NCBI database. The virus has been deposited in GenBank with the accession number MT259204.

The representative viruses in five families of mononegavirales were selected for multiple alignments and phylogenetic analysis based on RdRp sequences. Based on the RdRp conserved amino acid sequence of AhNsRV1 and the RdRp conserved amino acid sequences of 12 family *Mymonaviridae* viruses, multiple alignments showed that there were eight conserved motifs (Fig.S1).

Phylogenetic analysis showed that AhNsRV1 and some viruses of the family *Mymonaviridae* clustered into a single branch of the phylogenetic tree (Fig.2). In terms of genomic structure, AhNsRV1 is similar to Lentinus edodes negative-strand virus 1 (LeNsRV1) in the family *Mymonaviridae*, a known edible fungus Lentinus edodes. Therefore, AhNsRV1 is a new member of the family *Mymonaviridae* named Auricularia heimuer negative-stranded RNA virus 1 (AhNsRV1). Since there are few viruses in this family, there is no detailed classification. The continuous reports of negative-stranded RNA viruses suggest that the published negative-strand RNA viruses should be subdivided into at least two to three new genera according to the sequence length and genome structure.

This study isolated a novel mycovirus with a single-stranded negative strand RNA genome from *A. heimuer* strain CCMJ1222. To the best of our knowledge, this is the first report of single stranded negative strand RNA virus in *A. heimuer*. The research results of the virus enrich the resource bank of negative strand RNA virus in fungi.

Declarations

Acknowledgments: We gratefully acknowledge Qingcheng Liu and Guosheng Zhu from Guizhou Key Laboratory of Edible fungi breeding, Guizhou Academy of Agricultural Sciences, for providing us with their help. We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Author Contributions: Conceptualization, C. L., and Y. F.; Date curation, X. L and S. L.; Form analysis, X. L., and F. L. S., Funding acquisition, C, L.; Methodology, X. L.; Software, X. L., Supervision, C. L., and Y. F.; Validation, X. L., F. L. S .,Q. L., and G. Z.; Writing-original draft, X. L., Writing review& editing, X. L., F. L. S., C. L., Q. L., X. H., and G. Z. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards:

Funding: This research was funded by the National Key Research and Development Program of China (2021YFD1600401) and the Program of Introducing Talents of Discipline to Universities (No. D17014).

Conflicts of Interest: The authors declare no conflict of interest.

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

Informed consent: Informed consent was not required. The research involves no human participants or animals.

References

1. Pearson MN, Beever RE, Boine B et al (2009) Mycoviruses of filamentous fungi and their relevance to plant pathology. Mol Plant Pathol. 10: 115-128. doi: 10.1111/j.1364-3703.2008.00503.x.
2. Verma VC, Gange AC (2014) Entomopathogenic and nematophagous fungal endophytes. Advances in Endophytic Research 85-99. doi: 10.1007/978-81-322-1575-2_4.
3. Ghabrial SA, Castón JR, Jiang D et al (2015) 50-plus years of fungal viruses. Virology 479-480: 356-368. doi: 10.1016/j.virol.2015.02.034.
4. Sinden JW, Hauser E (1950) Report on two new mushroom diseases. Mushroom Science 1(1): 96-100.
5. Tavantzis SM, Romaine CP, Smith SH (1980) Purification and partial characterization of a bacilliform virus from *Agaricus bisporus*: A single-stranded RNA mycovirus. Virology 105(1): 94-102. doi: 10.1016/0042-6822(80)90159-2.
6. Grogan HM, Adie BAT, Gaze RH et al (2003) Double-stranded RNA elements associated with the MVX disease of *Agaricus bisporus*. Mycol Res. 107(2): 147-154. doi: 10.1017/s0953756203007202.

7. Elibuyuk IO, Bostan H (2010) Detection of a virus disease on white button mushroom (*Agaricus bisporus*) in Ankara, Turkey. Int J Agric Biol. 12: 597-600. doi: 10.1016/j.compag.2010.03.005
8. Won HK, Park SJ, Kim DK et al (2013). Isolation and characterization of a mycovirus in *Lentinula edodes*. J Microbiol. 51(1): 118-122. doi: 10.1007/s12275-013-2351-2.
9. Wang M, Liu X, Dai Y et al (2018) Phylogeny and species delimitation of Flammulina: taxonomic status of winter mushroom in East Asia and a new European species identified using an integrated approach. Mycol Prog. 17: 1013-1030. doi: 10.1007/s11557-018-1409-2.
10. Magae Y, Sunagawa M (2010) Characterization of a mycovirus associated with the brown discoloration of edible mushroom, *Flammulina velutipes*. Virol J. 7(1): 342. doi: 10.1186/1743-422X-7-342.
11. Chang Y, Chen J, Chang K et al (2019) Cloning and expression of the lectin gene from the mushroom *Agrocybe aegerita* and the activities of recombinant lectin in the resistance of shrimp white spot syndrome virus infection. Dev Comp Immunol. 90(02): 1-9. doi: 10.1016/j.dci.2018.07.020.
12. Amarasinghe GK, Ayllón MA, Bào Y et al (2019) Taxonomy of the order Mononegavirales: update 2019. Arch Virol. 164:1967–1980. doi: 10.1007/s00705-019-04247-4.
13. Jiāng D, Ayllón MA, Marzano SL (2019) ICTV Report Consortium. ICTV Virus Taxonomy Profile: *Mymonaviridae*. J Gen Virol. 100: 1343-1344. doi: 10.1099/jgv.0.001301.
14. Bolger AM, Lohse M, and Usadel B (2014) Trimmomatic: A flexible trimmer for illumina sequence data. Bioinformatics 30: 2114–20. doi: 10.1093/bioinformatics/btu170.
15. Yuan Y, Wu F, Si J et al (2019) Whole genome sequence of *Auricularia heimuer* (Basidiomycota, Fungi), the third most important cultivated mushroom worldwide. Genomics 111: 50-58. doi: 10.1016/j.ygeno.2017.12.013.
16. Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12: 357-60. doi: 10.1038/nmeth.3317.
17. Haas BJ, Papanicolaou A, Yassour M et al (2013) De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc. 8: 1494-512. doi: 10.1038/nprot.2013.084.
18. Fan X, Zhou Y, Xiao Y et al (2014) Cloning, expression and phylogenetic analysis of a divergent laccase multigene family in *Auricularia auricula-judae*. Microbiol Res 169(5-6): 453-62. doi: 10.1016/j.micres.2013.08.004.
19. Chiba S, Salaipeth L, Lin YH et al (2009) A novel bipartite double-stranded RNA mycovirus from the white root rot fungus *Rosellinia necatrix*: molecular and biological characterization, taxonomic considerations, and potential for biological control. J Virol. 83(24): 12801-12. doi: 10.1128/JVI.01830-09.
20. Darissa O, Willingmann P, Adam G (2010) Optimized approaches for the sequence determination of double-stranded RNA templatess. J Virol. Methods 169(2): 397-403. doi: 10.1016/j.jviromet.2010.08.013.

21. Marzano SL, Nelson BD, Ajayi-Oyetunde O et al (2016) Identification of diverse mycoviruses through metatranscriptomics characterization of the viromes of five major fungal plant pathogens. J Virol. 90: 6846-6863. doi: 10.1128/JVI.00357-16.
22. Guindon S, Delsuc F, Dufayard JF et al (2009). Estimating maximum likelihood phylogenies with phyML. Methods Mol Biol. 537: 113-137. doi: 10.1007/978-1-59745-251-9_6.
23. Guindon S, Dufayard JF, Lefort V et al (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 59: 307-21. doi: 10.1093/sysbio/syq010.
24. Larkin MA, Blackshields G, Brown NP et al (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947-8. doi: 10.1093/bioinformatics/btm404.
25. Kazutaka K, Standley DM (2016) A simple method to control over-alignment in the MAFFT multiple sequence alignment program. Bioinformatics 32: 1933-1942. doi: 10.1093/bioinformatics/btw108.

Figures

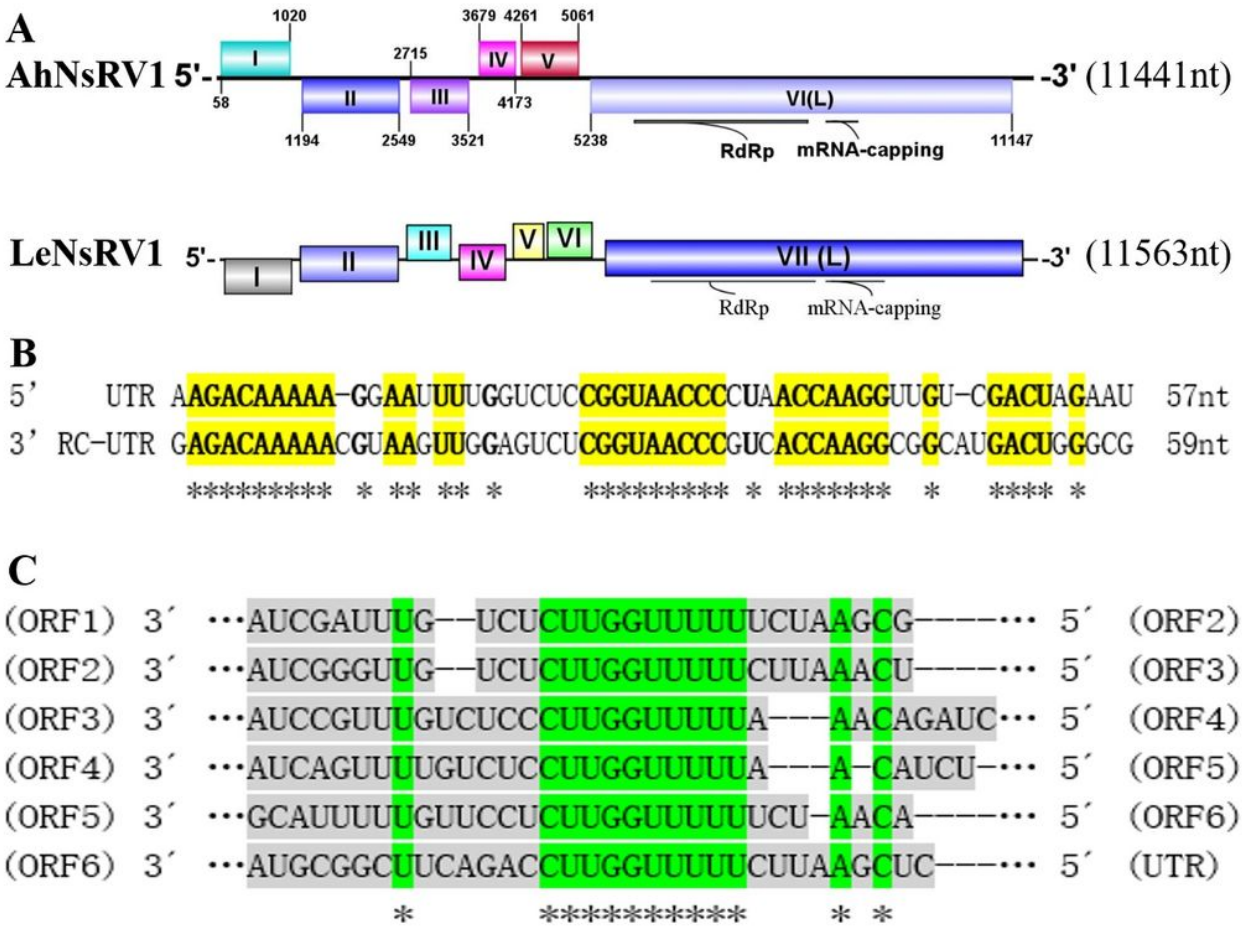

Figure 1

Structure and characteristics of the AhNsRV1 genome. (A) The comparison of the size and structure of AhNsRV1 and LeNsRV1 genomes. (B) The 3' UTR and 5' UTR reverse complementary sequences of AhNsRV1 are highly homologous, and the same amino acid is marked with a yellow background. (C) The multiple alignments of the sequence in the gene interval. The sequence direction is 3'-5', and the higher the degree of conservation of different colored epitopes, the deeper the color.

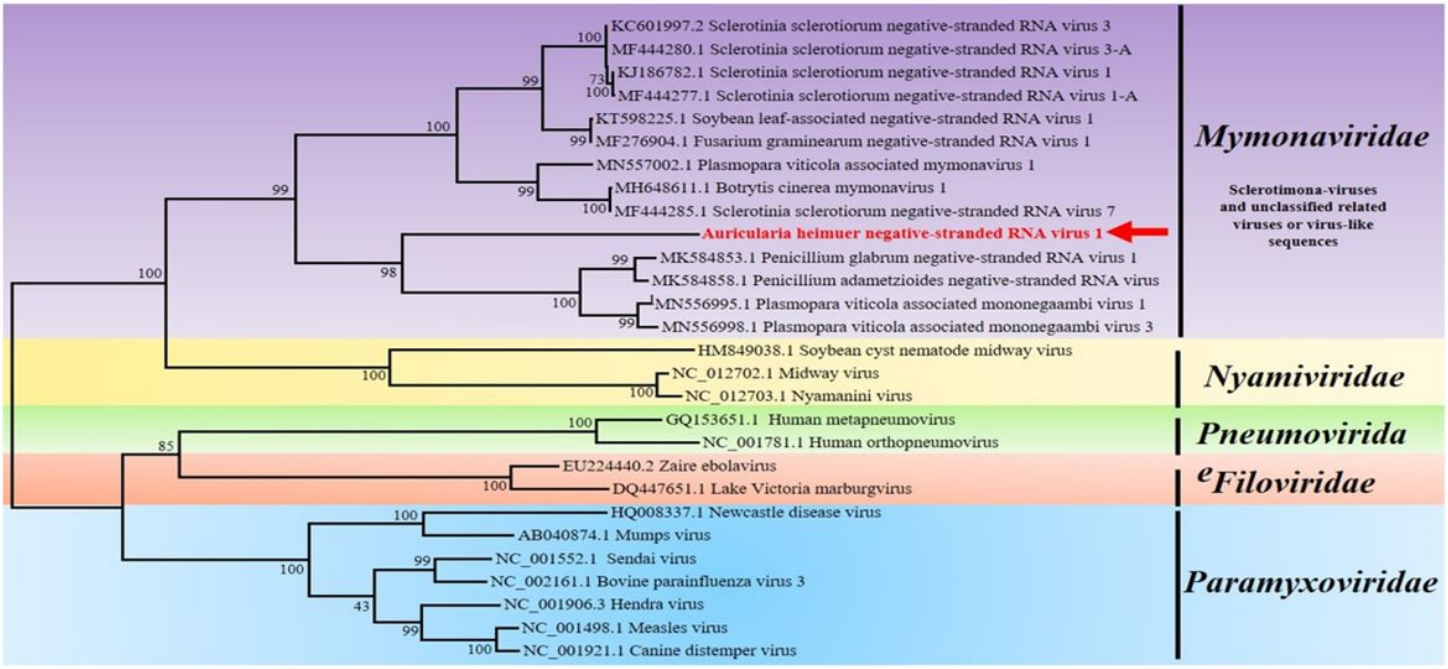

Figure 2

Maximum likelihood phylogenetic tree constructed based on the amino acid sequence of the RdRp conserved domain of the virus AhNsRV1 and the amino acid sequence of the RdRp conserved domain of several viruses in five families of Mononegavirales. The red arrow indicates the position of AhNsRV1 in the evolutionary tree.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AhNsRV1.fasta
- SupplementaryMaterials.doc