Prevalence of the root lesion nematode virus (RLNV1) in populations of Pratylenchus penetrans from North America

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Abstract

Root lesion nematode virus 1 (RLNV1) was discovered in the migratory endoparasitic nematode species Pratylenchus penetrans. It was found in a P. penetrans population collected from soil samples in Beltsville, Maryland, USA. In this study, the distribution of the RLNV1 in 31 geographically distinct P. penetrans populations obtained from different crops was examined. The results demonstrate that RLNV1 is widespread in North American populations of P. penetrans and exhibits low genetic variability in the helicase and RNA-dependent RNA polymerase regions of the genome.

Keywords

Distribution, Picorna-like virus, Pratylenchidae, Variability.

Presently, the number of viruses identified in the phylum Nematoda is limited. Considering the vast diversity of species within this phylum, more viruses naturally infecting nematodes are likely to be discovered. Recently, several new viruses were identified from wild populations of free-living (Félix et al., 2011; Franz et al., 2012; Frézal et al., 2019), animal-parasitic (Shi et al., 2016; Williams et al., 2019) and sedentary plant-parasitic nematodes (PPN; Bekal et al., 2011, 2014; Lin et al., 2018; Ruark et al., 2017, 2018).

We have recently discovered a new virus (the root lesion nematode virus, RLNV1) associated with the migratory nematode Pratylenchus penetrans (Vieira and Nemchinov, 2019). P. penetrans is an endoparasitic migratory PPN, which can infect a broad range of economically important crops (Castillo and Vovlas, 2007) and is among the top three most damaging species of PPN (Jones et al., 2013). Pratylenchus species were the most abundant PPN (69%) identified in 38,022 samples from the Pacific Northwest of North America by nematode diagnostic laboratories labs from 2012 to 2016 (Zasada et al., 2019).

The objectives of this study were: to determine the distribution of the RLNV1 in geographically distinct P. penetrans populations obtained from different North American cropping systems and to assess genetic variability of the virus by comparing sequence variations of the helicase and RNA-dependent RNA polymerase (RdRP) regions of identified RLNV1 isolates from the P. penetrans populations collected for this study.
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Material and methods

Collection of *Pratylenchus penetrans* isolates

A total of 31 populations of *P. penetrans* were used in this study from different geographic locations across Canada and USA (Table 1). Their identification was confirmed by morphological and molecular markers available for this species (Castillo and Vovlas, 2007; Peetz and Zasada, 2016). Most of the nematode populations were initially collected from different crops and maintained *in vitro* on sterilized corn roots (Vieira et al., 2015). The *P. penetrans* isolate NL 10p RH, from which RLNV1 (GenBank accession MK138531) was first identified, was used as positive control (Vieira and Nemchinov, 2019). For each population, several hundred nematodes were extracted from roots under intermittent mist for 5 d (Ayoub, 1980), washed three times in distilled water, frozen in liquid nitrogen, and stored at −80°C until subsequent analyses.

Nematode RNA extraction

Total RNA (50 ng per sample) was extracted from mixed life stages (eggs, second- to fourth-stage juveniles (J2-J4), adult females and males) of *P. penetrans* using the RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer’s instructions. RNA was treated with RNase-free DNase (QIAGEN) before reverse transcription. The quantity and quality of the extracted RNA was assessed using a ND-1000 NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and cDNA was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) following the manufacturer’s instructions. Primers were designed based on the available nucleotide sequence of the viral helicase (forward: 5’-GATCTCACGCGCTTTACCA-3’, pos. 4046-4064 and reverse: 5’-TCAGGTTCTGGAACAGGATTTC-3’, pos. 4978-4900) and RdRP (forward: 5’-CCCTATACAAATGGGAATAACAA-3’, pos. 7329-7353 and reverse: 5’-ATGCTCTCAAACCAGTCACTAT-3’, pos. 8307-8328) and used for PCR amplification of the corresponding sequence regions. The presence of the nematode transcripts within each generated cDNA library was confirmed by amplification of a 150 bp fragment from the 18 S rRNA gene (Fig. 1C). The RLNV1 was found in *P. penetrans* populations collected from potato, soybean, mint, apple and corn fields, while the virus was not detected in *P. penetrans* populations for 10 min. The PCR reactions contained 1×PCR buffer, 1 unit Taq Platinum polymerase (Invitrogen, Carlsbad, CA, USA) and 0.2 µM of each primer in a total of 50 µl of total solution. PCR products were separated by electrophoresis on a 1% agarose gel using TBE buffer (0.045 M Trisborate, 0.001 M EDTA, pH 8.0) and visualized using SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA, USA). The generated PCR products were then purified by PCR-purification kit (QIAGEN) and sequenced by Sanger sequencing using the corresponding forward and reverse primers by Macrogen Corp (Rockville, MD, USA).

Analysis of genetic diversity

Nucleotide sequences were aligned using MUSCLE program with default parameters incorporated into CLC Main Workbench software (V. 8). Predicted proteins sequences were obtained using CLC Main Workbench software (V. 8) and aligned using MUSCLE program with default parameters (Edgar, 2004). Pairwise genetic distances of both nucleotide and amino acid sequences (i.e. nucleotide differences and percent identity) were determined using CLC Main Workbench V. 8 (Qiagen, Hilden, Germany) software.

Results

Geographic distribution of RLNV1

A total of 31 populations of *P. penetrans* were assessed for the presence of the RLNV1 by RT-PCR with primers derived from helicase and RdRP regions of the virus genome (Fig. 1 and Table 1). These isolates were obtained from either established cultures or field collections of *P. penetrans*, originally collected from agricultural fields distributed throughout several areas of the USA and Canada (Table 1). *Pratylenchus penetrans* populations were collected mainly from potato and soybean fields, but were also collected from apple, cherry, corn, mint, and raspberry, which is consistent with the wide host range of this nematode species (Castillo and Vovlas, 2007).

PCR amplification resulted in amplicons of the expected sizes (855 bp for the helicase and RdRP regions of the virus genome (Fig. 1 and Table 1). These isolates were obtained from either established cultures or field collections of *P. penetrans*, originally collected from agricultural fields distributed throughout several areas of the USA and Canada (Table 1). *Pratylenchus penetrans* populations were collected mainly from potato and soybean fields, but were also collected from apple, cherry, corn, mint, and raspberry, which is consistent with the wide host range of this nematode species (Castillo and Vovlas, 2007).
Table 1. Results of the virus detection in different populations of *Pratylenchus penetrans*, and associated host plants.

| Genus         | Species    | Population | No. of individuals | Host     | Origin of population | Detection and sequencing |
|---------------|------------|------------|--------------------|----------|----------------------|-------------------------|
| *Pratylenchus*| *penetrans*| NL 10p RH  | Bulk               | Corn     | Beltsville, Maryland  | Yes*                    |
| *Pratylenchus*| *penetrans*| R3605      | Bulk               | Potato   | NA, Michigan         | –                       |
| *Pratylenchus*| *penetrans*| R3606      | Bulk               | Potato   | NA, Michigan         | Yes                     |
| *Pratylenchus*| *penetrans*| Greenhouse | 200                | Mint     | Various locations from OR and WA | Yes Yes |
| *Pratylenchus*| *penetrans*| Cherry     | 120                | Cherry   | Hood River, OR       | –                       |
| *Pratylenchus*| *penetrans*| CAR        | 200                | Apple    | Kenniwick, WA        | Yes Yes                |
| *Pratylenchus*| *penetrans*| Pole Road  | 200                | Raspberry| Lynden, WA           | –                       |
| *Pratylenchus*| *penetrans*| R-BDM      | Bulk               | Raspberry| Lynden, WA           | –                       |
| *Pratylenchus*| *penetrans*| 82–13      | Bulk               | Soybean  | Calumet Co., WI      | –                       |
| *Pratylenchus*| *penetrans*| 511–14     | Bulk               | Soybean  | Chippewa Co., WI    | Yes Yes                |
| *Pratylenchus*| *penetrans*| 546–16     | Bulk               | Soybean  | Chippewa Co., WI    | Yes Yes                |
| *Pratylenchus*| *penetrans*| 736–13     | Bulk               | Soybean  | Grant Co., WI       | Yes Yes                |
| *Pratylenchus*| *penetrans*| 92–16      | Bulk               | Soybean  | Iowa Co., WI        | –                       |
| *Pratylenchus*| *penetrans*| 99–16      | Bulk               | Soybean  | Lafayette Co., WI   | –                       |
| *Pratylenchus*| *penetrans*| 128–16     | Bulk               | Soybean  | Marathon Co., WI    | –                       |
| *Pratylenchus*| *penetrans*| 551–14     | Bulk               | Soybean  | Marquette Co., WI   | –                       |
| *Pratylenchus*| *penetrans*| Malek      | Bulk               | Potato   | Portage CO., WI     | Yes Yes                |
| *Pratylenchus*| *penetrans*| PRF        | Bulk               | Potato   | Portage CO., WI     | Yes Yes                |
| *Pratylenchus*| *penetrans*| PP6-1      | Bulk               | Potato   | Portage CO., WI     | –                       |
| *Pratylenchus*| *penetrans*| 469A-13    | Bulk               | Soybean  | Sheboygan Co., WI  | Yes Yes                |
| *Pratylenchus*| *penetrans*| Lauer      | Bulk               | Potato   | Waushara Co., WI   | Yes Yes                |
| *Pratylenchus*| *penetrans*| 422–14     | Bulk               | Soybean  | Wood Co., WI       | –                       |
| *Pratylenchus*| *penetrans*| PA         | Bulk               | Corn     | NA                   | Yes Yes                |
| *Pratylenchus*| *penetrans*| R3784      | Bulk               | Potato   | New Brunswick, CA** | No Yes                 |
| *Pratylenchus*| *penetrans*| R3790      | Bulk               | Potato   | New Brunswick, CA** | Yes Yes                |
| *Pratylenchus*| *penetrans*| R3794      | Bulk               | Potato   | New Brunswick, CA** | Yes Yes                |
| *Pratylenchus*| *penetrans*| R3813      | Bulk               | Potato   | New Brunswick, CA** | –                       |
| *Pratylenchus*| *penetrans*| R3816      | Bulk               | Potato   | New Brunswick, CA** | –                       |
| *Pratylenchus*| *penetrans*| R3739      | Bulk               | Potato   | Prince Edward Island, CA** | – – |
| *Pratylenchus*| *penetrans*| R3771      | Bulk               | Potato   | Prince Edward Island, CA** | – – |
| *Pratylenchus*| *penetrans*| QCLA       | Bulk               | Potato   | L’Acadie, Quebec, CA** | – – |

Notes: NA, not available specific location. *These sequences were original obtained and published in Vieira and Nemchinov (2019); **Canada; the symbol “−” denotes nematode populations with no detection regarding the presence of RLNV1.
obtained from raspberry and cherry (Table 1). Overall, the RLNV1 was detected in 45% of the \textit{P. penetrans} populations.

**Sequence variability within RLNV1 isolates**

The partial nucleotide sequences of the helicase and RdRP regions derived from 14 RLNV1 isolates associated with different \textit{P. penetrans} populations were obtained and compared between each other (Tables S1 and S2). In one population (\textit{P. penetrans} population R3784 collected from potato in New Brunswick, Canada), only the RdRP region was sequenced. Sequences exhibited a low level of nucleotide and amino acid variations in both regions of the viral genome. Pairwise comparisons of the nucleotide sequences in the helicase region ranged from 96.56 to 100%, while at the amino acid level the corresponding sequences had 98.89 to 100% identity among all the isolates (Table S1). For the RdRP fragment of the genome, the sequence identity ranged from 96.75 to 100% at the nucleotide level, and 98.11 to 100% at the amino acid level (Table S2). The Malek isolate originally collected from a potato field in Wisconsin displayed the highest genetic variability among all isolates.

**Discussion**

The main objective of this study was to characterize the extent of RLNV1 infection in \textit{P. penetrans} populations collected from different plant hosts across North America. We conclude that the virus is widespread in the USA and Canada and affects \textit{P. penetrans} populations collected from the diverse crop systems in North America. However, the virus was not found in \textit{P. penetrans} collected from 11 geographic locations (Fig. 1 and Table 1) and in the nematodes collected from raspberries and cherries (Table 1).

These findings may potentially indicate the presence of virus-free or virus-resistant \textit{P. penetrans} populations, especially in the case of positive and
negative results in nematodes collected from the same crop (potato and soybean) and in contiguous geographic locations. Negative results obtained with *P. penetrans* populations collected from raspberry and cherry may also suggest host-dependent susceptibility of *P. penetrans* to RLNV1. If true, this would likely be related to the genetic variability among the nematode isolates rather than to the virulence of RLNV1, which exhibited considerable homogeneity in the two regions examined in this study.

The observed prevalence of RLNV1 in populations of *P. penetrans* may also imply that this virus could represent a new resource as a potential biological control agent. Viruses associated with *C. elegans* (Orsay virus) and *C. briggsae* (Santeuil Le Blanc and Melnik viruses) were shown to infect intestinal cells, were horizontally transmitted, and slowed host progeny, thus affecting host fitness (Félix et al., 2011; Félix and Wang, 2019). While the interaction of RLNV with *P. penetrans* and its impact on the nematode’s viability and parasitism are not well understood, this approach may shed light on the potential avenue to reduce damage caused by *P. penetrans* to crop plants.

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**References**

Ayoub, S. M. 1980. Plant nematology, an agricultural training aid NemaAid Publications, Sacramento, CA.

Bekal, S., Domier, L. L., Niblack, T. L. and Lambert, K. N. 2011. Discovery and initial analysis of novel viral genomes in the soybean cyst nematode. Journal of General Virology 92:1870–9.

Bekal, S., Domier, L. L., Gonfa, B., McCoppin, N. K., Lambert, K. N. and Bhalerao, K. 2014. A novel flavivirus in the soybean cyst nematode. Journal of General Virology 95:1272–80.

Castillo, P. and Vovlas, N. 2007. *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management. Series: Nematology Monographs and Perspectives, Brill Leiden-Boston.

Edgar, R. C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5:113.

Félix, M. -A., Ashe, A., Piffaretti, J., Wu, G., Nuez, I., Bélicard, T., Jiang, Y., Zhao, G., Franz, C. J., Goldstein, L. D., Sanromán, M., Miska, E. A. and Wang, D. 2011. Natural and experimental infection of *Caenorhabditis* nematodes by novel viruses related to Nodaviruses. PLoS Biology 9:e1000586.

Félix, M.-A. and Wang, D. 2019. Natural viruses of *Caenorhabditis* nematodes. Annual Review of Genetics 53:313–26.

Franz, C. J., Zhao, G., Félix, M.-A. and Wang, D. 2012. Complete genome sequence of Le Blanc virus, a third *Caenorhabditis* nematode-infecting virus. Journal of Virology 86:11940.

Frézal, L., Jung, H., Tahan, S., Wang, D. and Félix, M.-A. 2019. Noda-like RNA viruses infecting *Caenorhabditis* nematodes: sympathy, diversity and reassortment. Journal of Virology 93: e017151-19.

Jones, J. T., Haegeman, A., Danchin, E. G., Gaur, H. S., Helder, J., Jones, M. G., Kikuchi, T., Manzalina-López, R., Palomares-Rius, J. E., Wesemael, W. M. and Perry, R. N. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology 14: 946–61.

Lin, J., Ye, R., Thkke-Veetil, T., Staton, M. E., Arelli, P. R., Bernard, E. C., Hewezi, T., Domier, L. L. and Hajimorad, M. R. 2018. A novel picornavirus-like genome from transcriptome sequencing of sugar beet cyst nematode represents a new putative genus. Journal of General Virology 99: 1418–24.

Peetz, A. B. and Zasada, I. A. 2016. Species-specific diagnostics using a β-1,4-endoglucanase gene for *Pratylenchus* spp. occurring in the Pacific Northwest of North America. Nematology 18: 1219–29.

Ruark, C. L., Koenning, S. R., Davis, E. L., Opperman, C. H., Lommel, S. A., Mitchum, M. G. and Sit, T. L. 2017. Soybean cyst nematode culture collections and field populations from North Carolina and Missouri reveal high incidences of infection by viruses. PLoS One 12:e0171514.

Ruark, C. L., Gardner, M., Mitchum, M. G., Davis, E. L. and Sit, T. L. 2018. Novel RNA viruses within plant parasitic cyst nematodes. PLoS One 13:e0193881.

Shi, M., Lin, X.-D., Tian, J.-H., Chen, L.-J., Chen, X., Li, C.-X., Qin, X.-C., Li, J., Cao, J.-P., Eden, J.-S., Buchénn, J., Wang, W., Xu, J., Holmes, E. C. and Zhang, Y.-Z. 2016. Redefining the invertebrate RNA virophere. Nature 540: 539–43.

Veiera, P. and Nemchinov, L. G. 2019. A novel species of RNA virus associated with root lesion nematode *Pratylenchus penetrans*. Journal of General Virology 100: 704–8.

Veiera, P., Eves-van den Akker, S., Verma, R., Wantoch, S., Eisenback, J. D. and Kamo, K. 2015. The *Pratylenchus penetrans* transcriptome as a source for the development of alternative control strategies: mining for putative genes involved in parasitism and evaluation of in planta RNAi. PLoS One 10:e0144674.

Williams, S. H., Che, X., Oleynik, A., Garcia, J. A., Muller, D., Zabka, T. S., Firth, C., Corrigan, R. M., Bries, T., Jain, K. and Wi, L. 2019. Discovery of two highly divergent negative-sense RNA viruses
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associated with the parasitic nematode, *Capillaria hepatica*, in wild *Mus musculus* from New York City. Journal of General Virology 100: 1350–62.

Zasada, I. A., Kitner, M. L., Wram, C., Wade, N., Ingham, R. E., Hafez, S., Mojtahedi, H., Chavoshi, S. and Hammack, N. 2019. Trends in occurrence, distribution, and population densities of plant-parasitic nematodes in the Pacific Northwest of the United States from 2012 to 2016. Plant Health Progress 20: 20–8.
Table S1. Nucleotide (A) and protein (B) pairwise comparisons of the helicase sequence data of different RLNV1 isolates from *Pratylenchus penetrans*.

|        | RLNV1 | 3606 | Greenhouse | Car | 546-16 | 736-13 | Malele | P* | PRF | 469A | Lauer | PA | R3794 | R3790 |
|--------|-------|------|------------|-----|--------|--------|--------|----|-----|------|-------|----|-------|-------|
| RLNV1  | 98.65 | 98.77| 99.26      | 98.53| 98.89  | 96.93  | 98.89  | 98.63| 98.89| 98.65| 99.75 | 98.65| 99.63 |
| 3606   | 11    | 99.39| 99.14      | 99.14| 99.51  | 96.56  | 99.51  | 99.02| 99.51| 99.75| 98.89 | 99.75| 99.02 |
| Greenhouse | 10 | 5    | 99.26      | 99.26| 99.63  | 96.93  | 99.63  | 99.14| 99.63| 99.39| 99.02 | 99.39| 99.14 |
| Car    | 6     | 7    | 6          | 99.26| 99.63  | 97.17  | 99.63  | 99.63| 99.63| 99.14| 99.51 | 99.14| 99.63 |
| 546-16 | 12    | 7    | 6          | 99.63| 96.44  | 99.63  | 98.89  | 99.63| 99.14| 98.77| 99.14 | 98.89|
| 736-13 | 9     | 4    | 3          | 3    | 3      | 96.81  | 100    | 99.26| 100  | 99.51| 99.14 | 99.51| 99.26 |
| Malele | 25    | 28   | 25         | 23   | 29     | 26     | 96.81  | 97.3 | 96.81| 96.56| 97.17 | 96.56| 97.3  |
| P*     | 9     | 4    | 3          | 3    | 3      | 0      | 26     | 99.26| 100  | 99.51| 99.14 | 99.51| 99.26 |
| PRF    | 3     | 8    | 7          | 3    | 9      | 6      | 22     | 6   | 99.26| 99.02| 99.88 | 99.02| 100   |
| 469A   | 9     | 4    | 3          | 3    | 3      | 0      | 26     | 0   | 6   | 95.51| 99.14 | 99.51| 99.26 |
| Lauer  | 11    | 2    | 5          | 7    | 7      | 4      | 28     | 4   | 8   | 4    | 98.89 | 99.75| 99.02 |
| PA     | 2     | 9    | 8          | 4    | 10     | 7      | 23     | 7   | 1   | 7    | 9     | 98.89| 99.88 |
| R3794  | 11    | 2    | 5          | 7    | 7      | 4      | 28     | 4   | 8   | 4    | 2     | 9    | 99.02 |
| R3790  | 3     | 8    | 7          | 3    | 9      | 6      | 22     | 6   | 0   | 6    | 8     | 1    | 8    |
### Table S1. (continued)

|      | RLNV1 | 3606 | Greenhouse | Car | 546-16 | 736-13 | Malele | P* | PRF | 469A | Lauer | PA | R3794 | R3790 |
|------|-------|------|------------|-----|--------|--------|--------|----|-----|------|-------|----|-------|-------|
| RLNV1| 99.63 | 100  |            | 100 | 99.26  | 100    | 100    | 100| 100 | 100  | 100    | 100 | 100   | 100   |
| 3606 | 1     | 99.63|            | 99.63| 98.89  | 99.63  | 99.63  | 99.63| 99.63| 99.63| 99.63  | 99.63| 99.63 | 99.63 |
| Greenhouse| 0 | 1    |            | 100 | 99.26  | 100    | 100    | 100| 100 | 100  | 100    | 100 | 100   | 100   |
| Car  | 0     | 1    | 0          | 99.26| 100    | 100    | 100    | 100| 100 | 100  | 100    | 100 | 100   | 100   |
| 546-16| 2     | 3    | 2          | 2   | 99.26  | 99.26  | 99.26  | 99.26| 99.26| 99.26| 99.26  | 99.26| 99.26 | 99.26 |
| 736-13| 0     | 1    | 0          | 0   | 2      | 100    | 100    | 100| 100 | 100  | 100    | 100 | 100   | 100   |
| Malele| 0    | 1    | 0          | 0   | 2      | 0      | 100    | 100| 100 | 100  | 100    | 100 | 100   | 100   |
| P*   | 0     | 1    | 0          | 0   | 2      | 0      | 0      | 100| 100 | 100  | 100    | 100 | 100   | 100   |
| PRF  | 0     | 1    | 0          | 0   | 2      | 0      | 0      | 0  | 100 | 100  | 100    | 100 | 100   | 100   |
| 469A | 0     | 1    | 0          | 0   | 2      | 0      | 0      | 0  | 0  | 100  | 100    | 100 | 100   | 100   |
| Lauer| 0     | 1    | 0          | 0   | 2      | 0      | 0      | 0  | 0  | 0    | 100    | 100 | 100   | 100   |
| PA   | 0     | 1    | 0          | 0   | 2      | 0      | 0      | 0  | 0  | 0    | 0      | 100 | 100   | 100   |
| R3794| 0    | 1    | 0          | 0   | 2      | 0      | 0      | 0  | 0  | 0    | 0      | 0   | 100   | 100   |
| R3790| 0    | 1    | 0          | 0   | 2      | 0      | 0      | 0  | 0  | 0    | 0      | 0   | 0     | 100   |

Note: Lower and upper panels represent the number of different nucleotides (counts) and nucleotide sequence similarity (%) within isolates, respectively.
Table S2. Nucleotide (A) and protein (B) pairwise comparisons of the RdRP sequence data of different RLNV1 isolates from *Pratylenchus penetrans*.

|       | RLNV1 | 3606 | Greenhouse | Car | 546-16 | 736-13 | Malele | P* | PRF | 469A | Lauer | PA | R3784 | R3790 | R3794 |
|-------|-------|------|------------|-----|--------|--------|--------|----|-----|------|-------|----|-------|-------|-------|
| RLNV1 | 99.16 | 99.16 | 98.74      | 98.95 | 98.85  | 97.9   | 98.85  | 99.69 | 98.95 | 99.27 | 99.79 | 99.43 | 98.85 | 98.64 | 98.64 |
| 3606  | 8     | 99.58 | 98.95      | 99.16 | 99.06  | 97.48  | 99.06  | 99.48 | 99.16 | 99.69 | 99.16 | 99.06 | 98.85 | 98.84 | 99.27 |
| Greenhouse | 8   | 4     | 99.37      | 99.58 | 99.48  | 97.48  | 99.48  | 99.48 | 99.58 | 99.48 | 99.16 | 98.85 | 98.84 | 98.85 | 99.06 |
| Car   | 12    | 10    | 6          | 99.79 | 99.69  | 97.06  | 99.69  | 99.06 | 99.79 | 98.85 | 98.74 | 98.22 | 98.22 | 98.43 | 98.43 |
| 546-16| 10    | 8     | 4          | 2    | 99.9   | 97.27  | 99.9   | 99.27 | 100  | 99.06 | 98.95 | 98.43 | 98.43 | 98.85 | 98.64 |
| 736-13| 11    | 9     | 5          | 3    | 1      | 97.17  | 99.79  | 99.16 | 99.9  | 98.95 | 98.85 | 98.32 | 98.32 | 98.53 | 98.53 |
| Malele| 20    | 24    | 24         | 28   | 26     | 27     | 97.17  | 98.01 | 97.27 | 97.38 | 97.69 | 96.75 | 97.17 | 96.96 | 96.66 |
| P*    | 11    | 9     | 5          | 3    | 1      | 2      | 27     | 99.16 | 99.9  | 98.95 | 98.85 | 98.32 | 98.32 | 98.53 | 98.53 |
| PRF   | 3     | 5     | 5          | 9    | 7      | 8      | 19     | 8    | 99.27 | 99.37 | 99.69 | 98.74 | 99.16 | 98.95 |       |
| 469A  | 10    | 8     | 4          | 2    | 0      | 1      | 26     | 1    | 7    | 99.06 | 98.95 | 98.43 | 98.43 | 98.64 |       |
| Lauer | 7     | 3     | 5          | 11   | 9      | 10     | 25     | 10   | 6    | 9    | 99.27 | 98.95 | 98.53 | 99.16 |       |
| PA    | 2     | 8     | 8          | 12   | 10     | 11     | 22     | 11   | 3    | 10   | 7    | 98.43 | 98.85 | 98.64 |       |
| R3784 | 15    | 9     | 11         | 17   | 15     | 16     | 31     | 16   | 12   | 15   | 10   | 15   | 99.37 | 99.79 |       |
| R3790 | 11    | 13    | 13         | 17   | 15     | 16     | 27     | 16   | 8    | 15   | 14   | 11   | 6     | 99.37 |       |
| R3794 | 13    | 7     | 9          | 15   | 14     | 14     | 29     | 14   | 10   | 13   | 8    | 13   | 2     | 6     |       |
Table S2. (continued)

|      | RLNV1 | 3606 | Greenhouse | Car 546-16 | 736-13 | Malele | P* PRF | 469A Lauer | PA | R3784 | R3790 | R3794 |
|------|-------|------|------------|------------|--------|--------|--------|------------|----|-------|-------|-------|
| RLNV1| 99.37 | 99.68| 99.68      | 99.68      | 99.37  | 99.37  | 99.37  | 99.37      | 100| 100   | 98.42 | 98.42 |
| 3606 | 2     |      |            |            |        |        |        |            |     |       |       |       |
| Greenhouse | 1 | 1 | 100 | 100 | 100 | 99.68 | 99.68 | 100 | 100 | 99.68 | 99.68 | 98.74 | 98.42 | 98.74 |
| Car  | 1 | 1 | 0 | 100 | 100 | 99.68 | 99.68 | 100 | 100 | 99.68 | 99.68 | 98.74 | 98.42 | 98.74 |
| 546-16 | 1 | 1 | 0 | 0 | 100 | 99.68 | 99.68 | 100 | 100 | 99.68 | 99.68 | 98.74 | 98.42 | 98.74 |
| 736-13 | 1 | 1 | 0 | 0 | 99.68 | 99.68 | 100 | 100 | 99.68 | 99.68 | 98.74 | 98.42 | 98.74 |
| Malele | 2 | 2 | 1 | 1 | 1 | 99.37 | 99.68 | 99.68 | 99.37 | 99.37 | 98.42 | 98.11 | 98.42 |
| P*   | 2 | 2 | 1 | 1 | 1 | 2 | 99.68 | 99.68 | 99.37 | 99.37 | 98.42 | 98.11 | 98.42 |
| PRF  | 1 | 1 | 0 | 0 | 0 | 1 | 100 | 99.68 | 99.68 | 98.74 | 98.42 | 98.74 |
| 469A | 1 | 1 | 0 | 0 | 0 | 1 | 100 | 99.68 | 99.68 | 98.74 | 98.42 | 98.74 |
| Lauer| 0 | 2 | 1 | 1 | 1 | 2 | 2 | 1 | 100 | 98.42 | 98.11 | 98.42 |
| PA   | 0 | 2 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 98.42 | 98.42 | 98.42 |
| R3784 | 5 | 5 | 4 | 4 | 4 | 5 | 4 | 4 | 5 | 5 | 99.68 | 100 |
| R3790 | 6 | 6 | 5 | 5 | 5 | 6 | 6 | 5 | 6 | 6 | 1 | 99.68 |
| R3794 | 5 | 5 | 4 | 4 | 4 | 5 | 5 | 4 | 4 | 5 | 5 | 0 | 1 |

Note: Lower and upper panels represent the number of different nucleotides (counts) and nucleotide sequence similarity (%) within isolates, respectively.