JANE Nerine potexvirus 1: a new Potexvirus species detected from Nerine in the United Kingdom

Beddoe, N., McGreig S, Walsh J, Fox A, Adams IP and Forsyth, A.

Title

Nerine potexvirus 1: a new potexvirus species detected from Nerine in the United Kingdom

Beddoe, N., McGreig, S., Walsh., J., Fox, A., Adams, I.P. and Forsyth, A.

Author information

Naomi Beddoe

ORCID 0000-0002-7766-891X

Canterbury Christ Church University, North Holmes Road, Canterbury, Kent CT1 1QU, UK

No working affiliation email address. Work completed when author was affiliated with this institution.

Sam McGreig

Sam.McGreig@fera.co.uk

ORCID – n/a

Fera Science Ltd., York Biotech Campus, Sand Hutton, York YO41 1LZ, United Kingdom.

John Walsh

John.Walsh@warwick.ac.uk

ORCID 0000-0002-7294-8701

School of Life Sciences, University of Warwick, Wellesbourne Campus, Warwick, CV35 9EF

Adrian Fox

Adrian.Fox@fera.co.uk

ORCID 0000-0003-1280-1836

Fera Science Ltd., York Biotech Campus, Sand Hutton, York YO41 1LZ, United Kingdom.

School of Natural and Environmental Sciences, Newcastle University, Agriculture Building, King's Road, Newcastle upon Tyne, NE1 7RU, United Kingdom.

Ian P. Adams

ORCHID: 0000-0002-5589-6460

Ian.Adams@Fera.co.uk

Fera Science Ltd., York Biotech Campus, Sand Hutton, York YO41 1LZ, United Kingdom.

Alec Forsyth – corresponding author

Alec.forsyth@canterbury.ac.uk

ORCID 0000-0002-4283-3583

Canterbury Christ Church University, North Holmes Road, Canterbury, Kent CT1 1QU, UK

Acknowledgements

This work was a collaboration between Canterbury Christ Church University and FERA. High-throughput sequencing was funded by DEFRA through the Euphresco VirusCurate project. Thanks to the Alan Brunt Collection at Warwick University for allowing access to the historic NVX samples.

Keywords

Potexvirus, Nerine, Hera, historic isolate, NVX, Illumina MiSeq

Main text

Title

Nerine potexvirus 1: a new potexvirus species detected from Nerine in the United Kingdom

Beddoe, N., McGreig, S., Walsh., J., Fox, A., Adams, I.P. and Forsyth, A.

Main text

The correct classification of a novel potexvirus sequence, identified from a Nerine plant in the UK, was possible due to the sequence comparison of contemporary field isolates with

newly generated sequence of historical isolates of nerine virus X (NVX), held in the Alan Brunt Collection at the University of Warwick.

Nerine virus X, is a species in the genus *Potexvirus*. The virus was originally described infecting *Nerine sarniensis* by Brunt *et al*. (1970) and later also inferred by particle morphology by electron microscopy (EM) in *Nerine* sp. from the Netherlands (Hakkaart *et al*.,1975). NVX was then identified in *Nerine* from the Netherlands using serological and EM methods (Maat, 1976) and in *Agapanthus* from England using EM and other biochemical techniques (Phillips & Brunt, 1980). Maat (1976) described two potexviruses in *Nerine*, for one he proposed the name nerine virus X (NVX), the other was identified serologically as narcissus mosaic virus (NMV). Hakkaart *et al*. (1975) reported that NVX often occurs in mixed infections with other viruses in *Nerine sarniensis*. Serologically indistinguishable isolates of NVX were identified in *Nerine sarniensis* (Brunt, 1977) and *Agapanthus africanus* (Phillips & Brunt, 1978). A potexvirus, serologically indistinguishable from NVX described by Brunt (1977) but showing distinct biological differences, was found in *Agapanthus praecox* subsp. *orientalis* (Phillips & Brunt, 1980). This isolate was more readily sap transmissible to other hosts and indicator plants, developed a higher concentration in indicator plants, and was more easily extracted and purified. This potexvirus was proposed to be the *Agapanthus* strain (NVX-A) of NVX. Brunt noted that NVX was common in some of the *Nerine* stocks tested (co-infecting with nerine latent virus (NeLV) and nerine yellow stripe virus (NeYSV) (Balasingham, 1989)).

Two complete NVX sequences from *Agapanthus* plants, one from Japan (NC_007679) and one from Taiwan (HQ166713) and two partial RNA-dependent RNA-polymerase (RdRp) sequences from *Nerine* (EF203683) and *Agapanthus* (EF203684) plants from the Netherlands were available on the NCBI database. Pairwise alignment of the RdRp sequences from *Agapanthus* plants showed that they shared between 97 and 99% nucleotide (nt) identity, yet the nt identity shared between *Agapanthus* and *Nerine* sequences dropped to 77%. These sequences were suggestive that two strains exist, the *Agapanthus* strain (NVX-A) and a *Nerine* strain (NVX-N), as previously proposed by Brunt.

Here we describe experiments by which we isolated the first full-length potexvirus sequence from *Nerine*. By using historical NVX sources from virus collections, we were able to determine that the virus identified in this study was a novel potexvirus and not a strain of NVX-N that had been previously identified by biological tests. A *Nerine sarniensis* x

bowdenii cv. Hera plant, that was growing in a pot was donated to the project as it showed potential virus-like (chlorotic) symptoms (Fig.1).

Fig.1 Symptomatic *Nerine sarniensis* x *bowdenii* cv. Hera leaves, showing mild chlorotic patches, suggestive of viral infection.

RNA was extracted from a fresh leaf using an RNeasy Plant Mini Kit (Qiagen) and cDNA was synthesised with an RNA to cDNA EcoDry Premix (Double Primed) kit (TakaraBio) following the manufacturer's instructions. Specific primers (NVX09/NVX10NEW, Supplementary 1) were designed based on the sequence of GenBank accession NVX-A isolate J (NC_007679.1) but did not amplify fragments, however, degenerate potexvirus primers (Supplementary 1, Potex-5 and Potex-1RC) designed to the RdRp region (Van der Vlugt & Berendsen, 2002) amplified fragments of the expected size of 737 bp. Purified (QIAquick PCR Purification Kit, Qiagen) fragments were ligated to pCR2.1 TA vector (Invitrogen) and sequenced by the Sanger method (DBS Genomics). The resulting sequence was similar (Blastn 97.61% nt identity over 13% coverage with e-value 0) to partial NVX-N IVT80054 RdRp sequence (EF203683.1). This suggested that there were some significant differences between NVX-A isolate J (NC_007679.1) and the potexvirus isolated from *Nerine* 'Hera' outside of the RdRp region. A MiSeq cDNA library was created from a dried leaf sample using the method described by Fowkes *et al*. (2021). The library was then sequenced and analysed. Reads were assembled using Angua version 3 (Fowkes *et al*. 2021), and a complete contig of a potexvirus-like sequence was produced. The coverage of the potexvirus was 1567x. The 6873 nt contig exhibited the expected ORFs associated with potexviruses and included a 17 nt 3' polyA. The MiSeq contig was validated by RT-PCR using primers (Supplementary 1, Nvx-mi-A-f/r, Nvx-mi-B-f/r, Nvx-mi-C-f/r, Nvx-mi-D-f/r, nvx.r.3.f.p1-p5, nvx.r.3.r.p1-p3, nvx.r.5.r.p1-p6) designed based on the MiSeq contig in overlapping regions, and the resulting amplicons were sequenced by the Sanger method (DBS Genomics). The 5' and 3' untranslated regions (UTR) were confirmed by RACE PCR (5' and 3' RACE PCR kit by TakaraBio) as per the manufacturer's instructions, using the primers shown in Supplementary 1 (nvx.gsp1, nvx.gsp2, nvx.gsp1.v2, nvx.gsp1.v3). The genome (6849 nt) organisation was typical of a potexvirus, including a 91 nt 5' UTR followed by 5 ORFs, replicase, triple gene block (TGB) 1-3, coat protein (CP) and a 125 nt 3' UTR (Fig.2). The 5' and 3' UTR lengths fell within the range of other potexvirus UTR lengths. The sequence was submitted to GenBank under accession number MZ643995.1 and is hereafter referred to as MZ643995.1 and/or nerine potexvirus 1 (NePV1).

Fig.2 Schematic representation of the NePV1 potexvirus genome identified from sequence obtained from *Nerine sarniensis* x *bowdenii* cv. Hera (MZ643995.1). Potexvirus-like regions were observed including a 5'untranslated region (UTR), replicase, triple gene block (TGB), coat protein (CP), 3'UTR and a poly(A) tail. Replicase; nt 92-4834 (1580 aa). TGB; nt 4867-6035. TGB1; 4867-5553 (228 aa), TGB2; nt 5540-5878 (112 aa), and TGB3; nt 5694-6035 (113 aa). CP; nt 6049-6723 (224 aa). The genome was 6849 nt long.

In addition to genome organisation the analysis of predicted ORFs also revealed the conserved amino acid (aa) motifs that would be expected within potexviruses, these are summarised in Supplementary 2. The potexvirus sequence described here shared similarities with the NVX-A genomes available on NCBI (NC_007679.1 and HQ166713.1). Pairwise alignment EMBOSS Stretcher (Madeira *et al*., 2022 available at https://www.ebi.ac.uk/Tools/psa/emboss_stretcher/) tool was used to calculate aa identities over the replicase and CP regions. NePV1 had significant sequence differences from the published NVX isolates derived from *Agapanthus*. It was not clear whether NePV1 represented a *Nerine* strain of NVX as distinct from the *Agapanthus* strain of NVX (NVX-A), as proposed by Brunt, or was a distinct and novel potexvirus. It was also not clear how previously reported NVX-A sequences (NC_007679.1, HQ166713.1) related to original NVX isolates and the previously proposed strains (NVX-N and NVX-A), as those original isolates had not been sequenced. To determine the relationship between NePV1 and the various *Nerine* potexvirus strains described and proposed by Brunt, Maat and others, we sequenced samples from an historical plant virus collection, with provenance to indicate these were related to the originally described isolates of NVX and agapanthus virus X.

Dried leaf samples were obtained from the Alan Brunt collection (University of Warwick) and two accessions, labelled NVX and agapanthus virus X were used. RNA extraction, library preparation and analysis were performed as described by Fowkes *et al*. (2021). The coverage of the NVX sequence from *Nerine* (submitted to GenBank, OQ731797) was 42672x and the coverage of the 'agapanthus virus X' sequence from *Agapanthus* (submitted to GenBank, OQ731796) was 4577x. Coverage figures were calculated using bwa mem 2, samtools and the BamToCov software (https://github.com/telatin/bamtocov). Sequence data from full-length sequences; NVX-A isolates (NVX-A isolate J, NC_007679.1 and NVX-A isolate AL1, HQ166713.1), NePV1 (MZ643995.1) and NMV (NC_001441.1) were compared to determine which sequences were most similar to the originally described NVX isolates NVX-N Brunt (OQ731797) and NVX-A Brunt (OQ731796).

The ICTV species demarcation criteria for the genus *Potexvirus* state that distinct species share < 72% nucleotide (or < 80% amino acid) identity over the replicase or CP regions (ICTV, 2022). The percentage aa and nt identity comparisons for the replicase (Table 1) and CP (Table 2) regions for the NVX isolates, NePV1 and NMV are shown in the tables below.

Table 1 Pairwise comparisons between the replicase nt and aa sequences of NVX isolates, NePV1 and NMV. Percentage nt identity is shown above the diagonal while aa identity is shown below. Virus isolates (with accession number) compared were: NVX-N Brunt (OQ731797), NVX-A Brunt (OQ731796), NVX-A isolate J (NC_007679.1), NVX-A isolate AL1 (HQ166713.1), NePV1 (MZ643995.1), NMV (NC_001441.1). The aa and nt sequences of the replicase for the four NVX isolates are highly similar and clearly distinct from the replicase sequences of NePV1 and NMV. The replicase of NePV1 is clearly distinct from that of NMV. Species differences are indicated by nt comparisons of < 72% and/or aa comparisons < 80% identity, and are shown in bold.

Replicase differences for demarcation between *Potexvirus* species are that they share < 72% nucleotide or < 80% amino acid identity (ICTV, 2022). The data presented in Table 1 suggest that NePV1 is a distinct virus from both NVX and NMV as both nt (~65% compared to NVX isolates and 53% compared to NMV) and aa identities (~67% compared to NVX isolates and ~41% compared to NMV) fall well below the threshold cutoff points. The NePV1 replicase protein consists of 1580 aa compared with 1504 aa of the four NVX isolates. Domain analysis using NCBI CDD (Marchler-Bauer A *et al*., 2015 available at https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) revealed a domain between the helicase and methyltransferase motifs in NePV1, which showed potential similarity (Bit Score: 67.63 E-value: 5.55e-11) to the Herpes virus major outer envelope glycoprotein (BLLF1), which equated to a 24.1% aa identity by pairwise analysis using EMBOSS Stretcher. This BLLF1 domain was not present in the replicase of the NVX isolates, nor in the replicase of the other 46 potexviruses (Supplementary 3) used in phylogenetic analysis (see below), suggesting that this domain may be unique to NePV1 within the potexviruses. Analysis of CP similarities is displayed in Table 2.

Table 2 Pairwise comparisons between the CP nt and aa sequences of NVX isolates, NePV1 and NMV. Percentage nt identity is shown above the diagonal while aa identity is shown below. Virus isolates (with accession number) compared were: NVX-N Brunt (OQ731797), NVX-A Brunt (OQ731796), NVX-A isolate J (NC_007679.1), NVX-A isolate AL1

(HQ166713.1), NePV1 (MZ643995.1) and NMV (NC_001441.1). The aa and nt sequences of the CP for the four NVX isolates were highly similar and clearly distinct from the CP sequence of NMV. The nt identity of the CP of NePV1 was clearly distinct from that of the four NVX isolates and of NMV. However, while the aa identity between NePV1 and NMV was clearly distinct, there was >80% similarity between the aa sequence of NePV1 and the four NVX isolates. Species differences are indicated by nt comparisons of < 72% and/or aa comparisons < 80% identity, and are shown in bold.

CP differences for demarcation between *Potexvirus* species are that they share < 72% nucleotide or < 80% amino acid identity (ICTV, 2022). The data presented in Table 2 suggest that NePV1 is a separate species from NVX, as although the aa identity is above the threshold value (~84-88%) when compared to the four NVX isolates, the nt identity of ~67% falls below the threshold criterion.

This combined comparative analysis of nt and aa identities of the replicase and CPs strongly suggests that NePV1 represents a distinct potexvirus and not an isolate/strain of NVX.

The relationship of NePV1 to other potexviruses was further investigated with two phylogenetic analyses. In the first analysis eight sequences were aligned using ClustalW. These sequences included NePV1, the four complete NVX sequences (NVX-A isolate J, NC_007679.1; NVX-A isolate AL1, HQ166713.1; NVX-N Brunt, OQ731797; NVX-A Brunt (OQ731796); and NMV (NC_001441.1), which had previously been described as infecting *Nerine* and had been identified in *Nerine* using serological assays (Matt, 1976). Potato virus x (PVX) NC_011620.1 was included as a potexvirus outlier and nerine yellow stripe virus (NeYSV) MT396083.1 was included as a non-potexvirus outlier. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei, 1993). A phylogenetic tree was generated using MegaX (Fig.3) and this analysis showed NePV1 as distinct from the four NVX isolates and from NMV.

Fig.3 Phylogenetic tree comparing four nerine virus X (NVX) sequences (NVX-A isolate J, NC_007679.1; NVX-A isolate AL1, HQ166713.1; NVX-N Brunt, OQ731797; NVX-A Brunt, OQ731796) with NePV1 (MZ643995.1) and narcissus mosaic virus (NMV, NC_001441.1). Potato virus x (PVX, NC_011620.1) was included as a potexvirus outlier and nerine yellow stripe virus (NeYSV, MT396083.1) was included as a non-potexvirus outlier. Sequences were aligned using ClustalW and the tree was generated using MegaX. The

analysis showed that NePV1 (MZ643995.1) sat alone, distinct from the four NVX sequences and NMV.

The phylogenetic analysis showed that 1) the original NVX-A Brunt (OQ731796) and NVX-N Brunt (OQ731797) viruses described by Brunt and sequenced from the historical isolates in the Brunt Collection shared a high similarity with the NVX-A isolates (NVX-A isolate J, NC_007679.1 and NVX-A isolate AL1, HQ166713.1) that appeared in the NBCI database from *Agapanthus*; 2) from the 4 sequences available to us there did not appear to be a unique *Agapanthus* strain sequence of NVX.

A further phylogenetic analysis was undertaken to investigate how NePV1 related to other potexviruses. Fifty-one full-length potexvirus sequences were aligned using ClustalW. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei, 1993). A phylogenetic tree was generated using MegaX. This analysis showed that NePV1 clustered with the NVX sequences, although it was distinct from them (Supplementary 4).

Complete potexvirus nucleotide sequences were tested for the presence of phylogenetic anomalies using the full suite of options in RDP4 with default parameters (Martin *et al*., 2015; Martin & Rybicki, 2000). No recombination events involving NePV1 or NVX were detected by four or more methods.

Summary

Many viruses were described in the pre-molecular age (Jones *et al*., 2020). The publication of the first, named, full-length genome on NCBI can become the *de facto* descriptor. There is no guarantee that this sequence is representative of the original viruses described, therefore obtaining data from historical collections can aid in taxonomic clarification.

Based on nt (~65%) and aa (~67%) identities of the replicase region, NVX from the Brunt collection and the newly identified and characterised potexvirus NePV1 are not the same species. NePV1 is distinct from the historical original NVX isolate and current NVX genomes and, as per the demarcation threshold for *Potexvirus* species (ICTV, 2022), could be classified as a novel species. The demarcation of a distinct *Potexvirus* species relies on one of four criteria being met based around nt or aa identities within the replicase or coat protein (ICTV, 2022). NePV1 meets 3 of these criteria to be classified as a distinct species from NVX.

Historical collections have enabled us to confirm that the complete NVX-A sequences (NC_007679.1 and HQ166713.1) in GenBank are NVX as described by Brunt (1977). Brunt described NVX in *Agapanthus* as being serologically indistinct from NVX from *Nerine*, but suggested the presence of *Nerine* and *Agapanthus* strains, based on infectivity assays. The data presented here suggests that these biologically distinct strains are genetically very closely related.

We have also been able to identify and characterise a novel potexvirus, which is not NVX or narcissus mosaic virus (NMV), which was also previously suggested (Maat, 1976) as a potexvirus that infected *Nerine* (*N. manselli*). We have tentatively named the newly described virus as NePV1, and propose a species name of *Potexvirus hera*.

The value of maintaining and sequencing historical collections has been clearly shown in this study, as access to the historical Alan Brunt collection has enabled clarification of the situation regarding NVX and potexvirus infection of *Nerine* and *Agapanthus*. The sequences of the historical isolates now provide information that can be related to the historically published virus biology. Furthermore, the ability to compare new sequence data to those from historical isolates has aided in the identification of a novel potexvirus, providing more data for those working in biosecurity to test for a virus that may be entering the country in plants or cut flowers.

References

Balasingham, G. (1989), ‘Purification, characterization and cDNA cloning of two filamentous viruses from Nerine’, Thesis, Massey University, New Zealand. Related publication, Balasingham, G., Ellison, N, Milne, K.S. and Forster, R.L.S. (1988), 'Sensitive and specific detection of two filamentous viruses from nerines using cloned cDNA probes’, Acta Horticulturae, 234(32).

Brunt, A. A., Hollings, M. and Stone, O. M., (1970). Rep. Glasshouse Crops Res. Inst. 1969: 138.

Brunt, A.A., (1977) Report of the glasshouse crops research institute for 1976, p. 123.

Fowkes, A.R., McGreig, S., Pufal, H., Duffy, S., Howard, B., Adams, I.P., Macarthur, R., Weekes, R. and Fox, A. (2021), ‘Integrating High throughput Sequencing into Survey Design

Reveals Turnip Yellows Virus and Soybean Dwarf Virus in Pea (Pisum Sativum) in the United Kingdom', Viruses, 13(12), p. 2530.

Hakkaart, F.A., Maat, D.Z. and Quak, F. (1975), 'Viruses and meristem culture of Nerine', Acta Horticulturae, 47, pp. 51-54, https://doi.org/10.17660/ActaHortic.1975.47.6

ICTV, 2022 Genus: Potexvirus https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/alphaflexiviridae/1330/genus-potexvirus Accessed 28 October 2022.

Jones, R.A.C., Boonham, N., Adams, I.P., Fox, A. 2020, 'Historical virus isolate collections: An invaluable resource connecting plant virology's pre-sequencing and post-sequencing eras', Plant Pathology, https://doi.org/10.1111/ppa.13313

Maat, D.Z. (1976) 'Two potexviruses in Nerine', Netherlands Journal of Plant Pathology, 82, pp. 95–102, https://doi.org/10.1007/BF01976692

Madeira F, Pearce M, Tivey ARN, et al. Search and sequence analysis tools services from EMBL-EBI in 2022. Nucleic Acids Research. 2022 Jul;50(W1):W276-W279. DOI: 10.1093/nar/gkac240. PMID: 35412617; PMCID: PMC9252731

Marchler-Bauer A et al. (2015), "CDD: NCBI's conserved domain database.", Nucleic Acids Res.43(D)222-6.

Martin, D. and Rybicki, E. (2000) 'The RDP method: RDP: detection of recombination amongst aligned sequences', Bioinformatics, 16, pp. 562–563.

Martin, D.P., Murrell, B., Golden, M., Khoosal, A., Muhire, B. (2015) 'RDP4: detection and analysis of recombination patterns in virus genomes', Virus Evolution, 1, 003. https://doi.org/10.1093/ve/vev003

Phillips, S. and Brunt A.A. (1980) 'Some hosts and properties of an isolate of nerine virus x from Agapanthus praecox subsp. orientalis', Acta Horticulturae, 110, pp. 65-70.

Phillips, S. and Brunt, A.A. (1978) 'Bulbs crops: Nerine', Report of the glasshouse crops research institute for 1977. p. 131.

Tamura K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526.

Van der Vlugt, R.A.A and Berendsen, M. (2002), 'Development of a General Potexvirus Detection Method', European Journal of Plant Pathology, 108(4), pp. 367–371.

Abstract

We identified full-length sequence of a potexvirus infecting *Nerine* plants in the UK. By sequencing viruses in historical collections we have been able to demonstrate that the identified virus is a potentially novel species, for which we propose the name nerine potexvirus 1. Analysis of the potexviruses in the historic collections has also enabled us to generate the first full length sequence of nerine virus X (NVX) to be isolated from *Nerine*; and to clarify the taxonomy of NVX isolates infecting *Nerine* and *Agapanthus*. Analysis of isolates from the historical collections has enabled us to link biological data gathered in the pre-genomic era to specific isolate sequences.

Table 1

	NePV1	NVX-N Brunt	NVX-A Brunt	NVX-A isolate J	NVX-A isolate AL1	NMV	
NePV1	X	**65.5**	**65.6**	**65.6**	**65.6**	**53**	Nucleotide
NVX-N Brunt	**67.2**	X	99.9	99	98.6	**53.6**	
NVX-A Brunt	**67.2**	99.9	X	99	98.7	**53.5**	
NVX-A isolate J	**67**	99.1	99.1	X	98.7	**53.3**	
NVX-A isolate AL1	**66.9**	98.5	98.5	98.5	X	**53.3**	
NMV	**41.5**	**43.2**	**43.2**	**43.2**	**43.0**	X	
	Amino acid						

Table 2

	NePV1	NVX-N Brunt	NVX-A Brunt	NVX-A isolate J	NVX-A isolate AL1	NMV	
NePV1	X	**67.4**	**67.3**	**67.4**	**67.1**	**53.7**	Nucleotide
NVX-N Brunt	87.7	X	99.8	99.7	99	**49.5**	
NVX-A Brunt	87.7	100	X	99.5	98.9	**49.4**	
NVX-A isolate J	87.7	100	100	X	99	**49.5**	

NVX-A isolate AL1	84.2	96.1	96.1	96.1	X	**49.5**	
NMV	**35.8**	**35.5**	**35.5**	**35.5**	**33.9**	X	
	Amino acid						

Figure 1

Figure 2

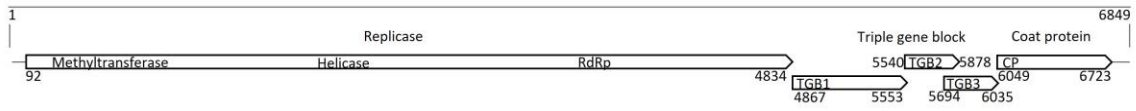
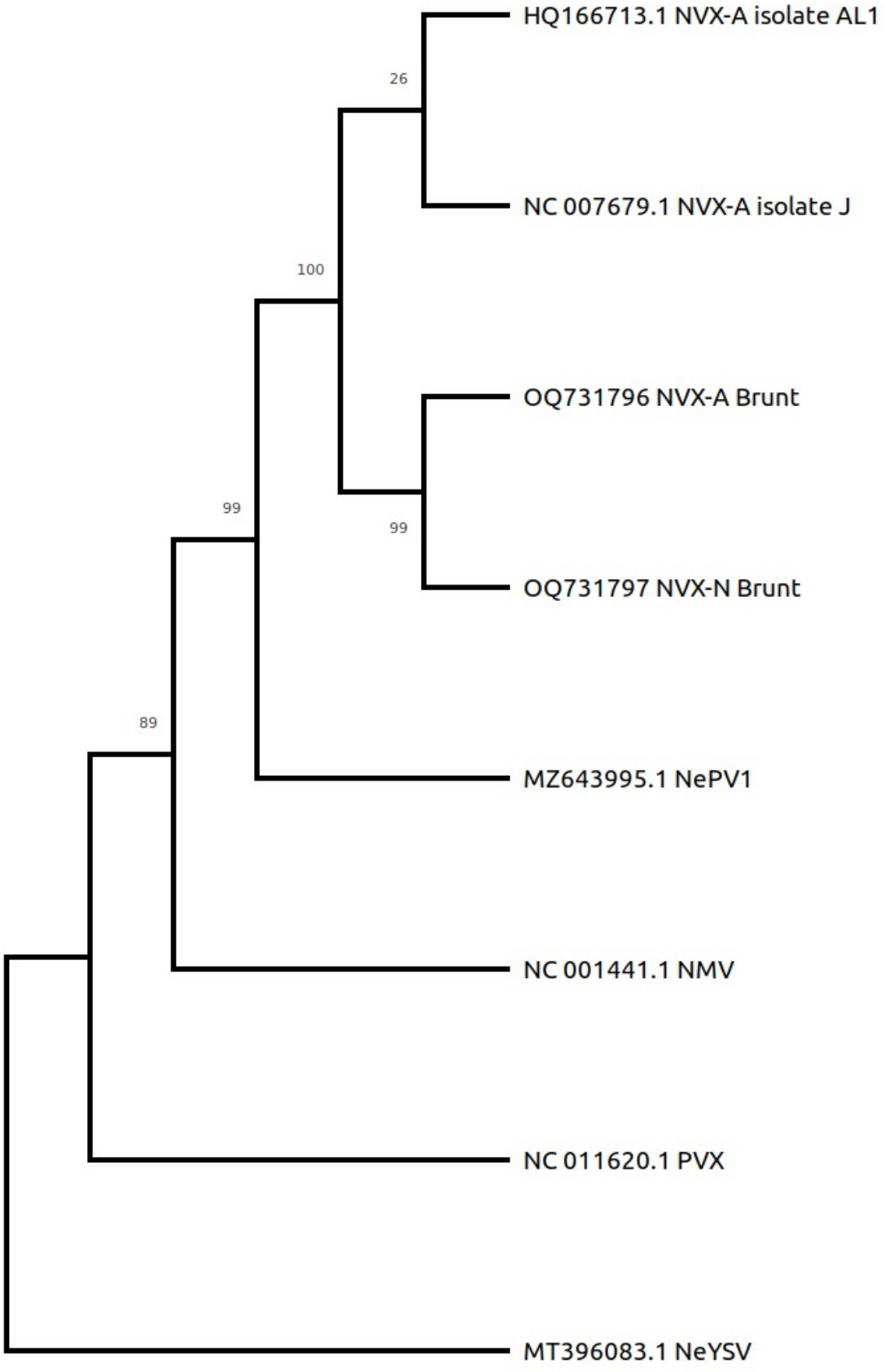


Figure 3


HQ166713.1 NVX-A isolate AL1
26
NC 007679.1 NVX-A isolate J
100
OQ731796 NVX-A Brunt
99
99
OQ731797 NVX-N Brunt
89
MZ643995.1 NePV1
NC 001441.1 NMV
NC 011620.1 PVX
MT396083.1 NeYSV

327