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ORIGINAL ARTICLE

A new virus, classifiable in the family *Tombusviridae*, found infecting *Solanum tuberosum* in the UK

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Abstract

A novel virus was discovered in a freeze-dried collection held at SASA, UK, originating from potato (*Solanum tuberosum*) cv. Nadine. The complete sequence of the viral RNA was determined to be 3674 nucleotides in length encoding five predicted proteins. Based on the deduced genome organization and phylogenetic analysis, this virus represents a putative new member of the genus *Alphanecrovirus,* family *Tombusviridae*, most closely related to isolates of *Olive mild mosaic virus*. The virus was easily transmitted to indicator plants with symptoms that were slower to develop and less severe than those of related viruses. To distinguish this virus, the clearest symptom differences occurred with *Nicotiana debneyi*, *Chenopodium amaranticolor* and *Ch. quinoa*. The virus was detected with antisera to the related viruses tobacco necrosis virus A and tobacco necrosis virus D. The close association to the tobacco necrosis viruses would suggest this virus is not a new introduction to potato but in the past has been misidentified as one of these viruses. The virus isolate has been named potato necrosis virus.

Introduction

In recent years the family *Tombusviridae* has undergone a reorganisation and introduction of new genera [20, 22]. The genus necrovirus is now obsolete and the seven members have been reassigned. *Olive latent virus 1*, *Olive mild mosaic virus* and *Tobacco necrosis virus A* form the genus *Alphanecrovirus*, whereas *Beet black scorch virus*, *Leek white stripe virus* and *Tobacco necrosis virus D* form the genus *Betanecrovirus*. The lack of sequence data for *Chenopodium necrosis virus* has reduced its status to an unassigned tombusvirid.

Genome organisation and expression for members of the alpha and betanecroviruses (necrovirids) are similar to other genera in the *Tombusviridae*, particularly the carmoviruses. The necrovirids have monopartite, positive sense, linear RNA genomes that code for five or six proteins, the 5' end of the RNA is not capped and the 3' end does not have a poly (A) tail. All code for a pre-readthrough (pRT) RNA dependent RNA polymerase (RdRp), with the full length RdRp generated from a readthrough (RT), amber stop codon (UAG). The pRT protein, as well as the full-length RdRp is essential for RNA replication in olive latent virus 1 (OLV1) and tobacco necrosis virus D (TNVD) [19]. Apart from encapsidation, the coat protein (CP) of these viruses have been reported to interact with the zoospores of the fungal vector *Olpidium brassicae* [4]. Small open reading frames (ORFs) encode for movement proteins (MP1 and MP2), but individual viruses classified in these genera may have extra ORFs not shared by all members of the genus.

The necrovirids are transmitted in the soil to host roots by the fungal pathogen *Olpidium brassicae* [26], although OLV1 appears to be transmitted through soil in the absence of vectors [13]. These viruses are easily transmitted to indicator plants by mechanical inoculation but are not transmitted by seed or pollen [3]. The only member of the necrovirids reported to infect potato naturally is TNVD [16]. TNVD is reported to have a large host range spanning more than nine families including potato, olive and many other crop species, some of which become systemically infected. In potato the disease is rare but it may cause significant tuber blemishes of dark brown lesions with radial or reticular cracks on harvested tubers. During storage, blisters may develop, which later become sunken. The sunken lesions may cover most of the tuber surface [16]. The natural host range of tobacco necrosis virus A (TNVA) is reported as limited and no information is available on whether this virus

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naturally infects potato. The experimental host range for TNVA is broad with more than 30 families, of which most only produce local lesions [16]. Olive mild mosaic virus (OMMV) has been found in such diverse species as olive [6], spinach [15], and tulip. OLV1 has been found in tomato [1], olive [10, 14, 18] and tulip [17]. In this study the complete genome of a new classifiable member of the genus *Alphanecrovirus* is presented. The molecular and biological characteristics of the virus are compared with related viruses and the diagnostic implications discussed.

Materials and methods

Virus isolates used in this study came from virus collections at SASA, the Pacific Agri-Food Research Centre, Canada and the Plant Protection Service, The Netherlands (Table 1).

Indicator plants and growth conditions

Plants were grown under artificial light to give a 16 h day length with temperatures of 20 °C by day and 15 °C at night. Plants were inoculated mechanically by rubbing carborundum dusted young leaves with virus infected sap extracted 1:5 in water. Indicator plants including *Nicotiana* and *Chenopodium* species, *Solanum melongena* (aubergine) and *Cucumis sativus* (cucumber) were grown from seed. Potato cultivars (cvs) were grown from pathogen-tested *in vitro* plantlets obtained from SASA's nuclear stock collection. Symptoms were recorded as they developed on inoculated plants. Where symptom differences occurred with different viruses, the inoculations were repeated for confirmation.

DAS-ELISA

DAS-ELISA was performed with antisera to TNVA and TNVD from DSMZ, Germany (Cat No. AS-0208 and AS-0168). Plant sap was extracted from virus infected and uninfected *N. benthamiana* plants. Measurements at OD_{405nm} were taken at 2 hours and overnight and recorded as an average of two readings from duplicate samples. A positive result was reported if above the threshold of twice the OD_{405nm} value of a non-infected plant.

RNA extraction and analysis

Viral RNA was extracted from freeze dried or fresh plant leaves using the RNeasy kit (Qiagen, cat. 74104). Reverse transcriptase PCR (RT-PCR) was performed using the Jumpstart Readymix (Sigma, cat. P0982), in a one-step reaction with primers at a concentration of 10 pmol/µl. The typical thermal cycler program consisted of an initial reverse transcriptase step of 48 °C for 20 min and denaturation at 94 °C for 2 min followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 52 °C for 30 sec and extension at 72 °C for 1 min, followed by 72 °C for 7 min. The primer set TNVA 760F/ TNVA 1637R (this study) was used to verify infection of potato necrosis virus (PoNV), OMMV and TNVA and primer set TNVD-DF/ TNVD-DR [5] was used to verify infection of TNVD.

Genome sequencing and analysis

An initial partial sequence of PoNV was produced with primers designed to amplify part of the RdRp gene of TNVA (primers TNV-760F 5'-AAC AGC GTC AGT TGT TAC GCT A-3' and TNV-1637R 5'-TTC TCA CAG ACA ATG ACG CAG T-3'). The product was sequenced directly and specific primers designed and used in RT-PCR reactions with further primers designed to the TNVA genome (not shown). This technique was repeated for the length of the genome to give a series of overlapping products of approximately 600 bp length. At least two products were produced that cover each region of the viral genome. The 5' end of the genome was confirmed through the use of the 5' RACE system (Invitrogen cat. 18374058), using the specific primers PoNV-230R (5'-CTT CTG GGA CAA CCT TAG TGC GCT-3') and PoNV-200R (5'-TTC CTC AAA GCA CTC GTT GAT GT-3') in a nested PCR with primers provided with the kit. The 3' end of the genome was confirmed by the addition of a polyA tail using the epicentre kit (Illumina cat. no. PAP5104H) followed by nested PCR using the specific

Table 1 Virus isolates used during this study

Virus	SASA designation	Collection	Source plant	Other designation
TNVA	QV322	SASA, UK	UK origin, soil bait test, from narcissus site	
TNVA	QV333	Canadian Plant Virus Collection	*Phaseolus vulgaris* cv. Kidney bean Canadian origin, collected before 1996	CPVC ID 811
TNVD	QV330	The Netherlands	*Cucumis sativus*	21000015
OMMV	QV332	Canadian Plant Virus Collection	*Nicotiana tabacum* cv. Samsun, collected before 1996	CPVC ID 275
PoNV	QV323	SASA, UK	*Solanum tuberosum* cv. Nadine, UK origin, collected 2005	Isolate 298 SASA Virology Section

primers PoNV-2906F (5'-TCA GCA TTA GGT GCG TTT AGC TT-3') and PoNV-3007F (5'-ATC CTG TTC AGT CAT CTA TAT ACC TA-3') with Not1pdT designed for the polyA tail (5'-ACT GGA AGA ATT GGC GGC CGC AGG AAT TTT TTT TTT-3').

Encoded proteins were identified from the translation of the nucleotide sequence and similarities to related viral sequences from the NCBI database. Alignments for nucleotide and encoded protein sequences were performed with related viruses using the clustal W algorithm. Sequence identities were calculated using the Megalign program (lasergene 11, DNASTAR) and phylogenetic trees were generated with the MEGA 5 program [24] using the neighbor-joining method (replicas bootstrapped to 1000).

Results

At SASA in 2005, virus testing of potato for the tobacco necrosis viruses relied on the use of indicator plants (*Ch. quinoa, N. tabacum* cv. White Burley and *N. debneyi*). Symptoms produced with the tubers of a commercial locally grown potato crop (cv. Nadine), suggested TNV was present. The leaf material of symptomatic *Ch. quinoa* was stored in the virus collection as a freeze-dried sample until it was used as an isolate for validating new molecular assays for TNV. The sequence of PCR products, revealed this sample to contain a virus that was related to but distinct from members of the genus *Alphanecrovirus*.

Molecular analysis

The complete RNA genome comprised 3674 nucleotides (nts) (GenBank, KP901095) and was shorter than genome lengths previously recorded for a necrovirid (Table 2). The viral RNA codes for five predicted ORFs (Fig 1), with the same genome organization previously described for OLV1 and OMMV [6, 18] (Fig 1). ORF1 follows a 5' untranslated region of 62 nts. The first AUG codon is at position 63-65 nts and extends to a UAG amber termination codon at position 669-671 nts and this encodes the pre-read-through RdRp protein of 23 kDa (p23). The read-through termination codon gives ORF2 (63-2237 nts) the full-length RdRp of 82 kDa. The RdRp overlaps with ORF3, MP1 a small 8kDa protein (2221-2442 nts), followed by ORF4, MP2 a 6kDa protein (2442-2612 nts). The last adenine base of the MP1 stop codon is the first base of the MP2 start codon (nucleotide 2442). MP2 has a predicted transmembrane motif

Table 2 Sequence identity of PoNV (KP901095) with related viruses classifiable in the *Tombusviridae*

Genus of the *Tombusviridae*	Virus	Viral genome sizes (nt) (NCBI database)	% Sequence identity complete genomes (nt)	% Sequence identity for encoded proteins			
				RdRp	MP1	MP2	CP
Alphanecrovirus	TNVA	3682-3684	73	86	72	95	49
	OMMV	3683	77	88	71	96	76
	OLV1	3699-3702	74	86	66	96	40
Betanecrovirus	TNVD	3761-3762	50	35	23	16	78
	LWSV	3662	43	35	22	19	26
	BBSV	3642-3644	45	36	23	26	40
Macanavirus	FNSV	3966	50	52	48	56	21
Gallantivirus	GaMV	3803	49	51	45	36	21
Betacarmovirus	TCV	4053-4054	42	38	n/a	n/a	18
Gammacarmovirus	MNSV	4261-4323	42	39	n/a	n/a	20

Comparisons were performed for the complete viral genomes and encoded proteins. The compared viruses were tobacco necrosis virus A (TNVA, M33002); olive mild mosaic virus (OMMV, AY616760); olive latent virus 1 (OLV1, X85989); tobacco necrosis virus D (TNVD, U62546); leek white stripe virus (LWSV, X94560); beet black scorch virus (BBSV, AF452884); furcraea necrotic streak virus (FNSV, FJ768020); galinsoga mosaic virus (GaMV, Y13463); turnip crinkle virus (TCV, M22445) and melon necrotic spot virus (MNSV, M29671). n/a = not applicable

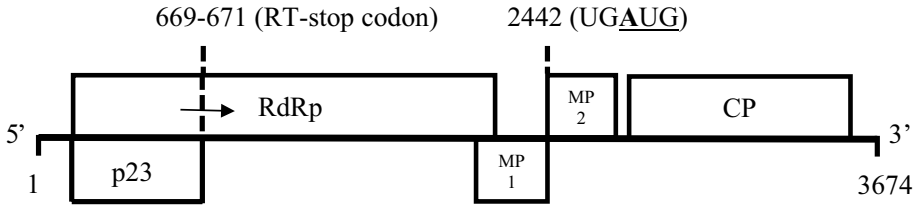

Fig. 1 Schematic representation of the viral genome of potato necrosis virus with the five predicted ORFs

(AILLLILAILVV) a possible membrane docking site, as suggested for Galinsoga mosaic virus [29]. A similar motif has been found in OLV1 (AILILILAILVV) [8] and OMMV (AILILILAILVV) according to available sequences (GenBank AY616760). The fifth protein (2632-3435 nts) encodes the 29 kDa CP. The CP has the 'S' signature consensus motif of 26 conserved amino acids (aa) [FYW]-x-[PSTA]-x(7)-G-x-[LIVM]-x-[LIVM]-x-[FYWIL]-x(2)-D-x(5)-P [9, 12], this virus has the same motif as OMMV and TNVD (Y-x-P-x(7)-G-x-V-x-M-x-I-x(2)-D-x(5)-P). The CP is followed by the 3' untranslated region (3'UTR). As with related viruses a predicted cap-independent translation element (TE) can be found within the 3'UTR; this is a conserved 18 nt sequence (CGGAUCCUGGGAAACAGG) at position 3493-3510. The virus was tentatively named potato necrosis virus and given the designation in SASA's virus collection QV323 (PoNV-QV323)

The sequence identities between PoNV and related viruses are shown in Table 2 and the phylogenetic analyses of encoded viral proteins in Figs. 2. The complete genome sequence confirmed OMMV as the most closely related virus (77% nt). The phylogenetic analysis of the RdRp (Fig. 2a), MP1 (Fig. 2b) and MP2 (Fig. 2c), shows PoNV forming distinct clades with TNVA, OLV1 and OMMV (alphanecroviruses), with high sequence identity confirmed in Table 2. In addition the alphanecroviruses share a closer relationship with the two monotypic tombusvirids, Furcraea necrotic streak virus (FNSV) and Galinsoga mosaic virus (GaMV) than the betanecroviruses for the RdRp and MPs (Fig 2a, b, c). However, the CP of PoNV shares high identity with TNVD (a betanecrovirus) and OMMV (78% and 76% aa) whilst OMMV and TNVD CPs are more closely related (83% aa). The phylogenetic tree for the CPs (Fig. 2d) showed TNVD, OMMV and PoNV forming a distinct clade, with the other necrovirids (OLV1, TNVA, BBSV, LWSV) having a less defined relationship with both these viruses and each other.

Symptoms on plants

Attempts to inoculate PoNV-QV323 from indicator plants to potato plants were unsuccessful. PoNV-QV323 was maintained in *N. benthamiana* and sap-inoculated on two occasions to four plants of each potato cv. Nadine, Culster, Carnival and Marfona. No cv. produced any symptoms and RT-PCR of the inoculated leaves confirmed the plants to be uninfected.

To examine infection and symptom development on common indicator plants the initial experiment compared PoNV-QV323 with TNVA-QV322. Each virus was inoculated to two plants of *N. benthamiana, N. bigelovii, N. clevelandii, N. debneyi, N. occidentalis, N. tabacum* cv. White Burley, *Ch. amaranticolor, Ch. murale, Ch. quinoa, S. melongena* and *C. sativus*. The *S. melongena* and *C. sativus* showed no symptoms with either virus and subsequent RT-PCR confirmed they were not infected. The majority of indicator plants showed symptoms only on the inoculated leaves with similar symptoms displayed by both viruses. Differences were observed with the severity of symptoms and their speed of development, with PoNV being less aggressive than TNVA. Further inoculation studies included TNVA-QV333, OMMV-QV332 and TNVD-QV330 (Table 1) to both *Nicotiana* and *Chenopodium* species, with symptoms recorded on a daily basis. The two TNVA isolates exhibited identical symptoms on the different indicator plants. Table 3 contains a summary of symptoms on the inoculated leaf of different indicator plants by different viruses. Symptoms at 2, 3, 4 and 7 days post inoculation (dpi) are presented, the development of systemic symptoms are also noted.

Chenopodium spp

Ch. amaranticolor can be used to distinguish PoNV from related viruses. TNVA, TNVD and OMMV produced symptoms of chlorotic spots 2-3 dpi that developed into necrosis by 4 dpi and 'paper thin' necrosis after 7 dpi. PoNV symptoms developed much later with chlorotic spots not appearing until 6-7 dpi that did not turn necrotic (Fig. 3).

Ch. murale inoculated with TNVD showed small necrotic pitting 2 dpi that developed into necrotic lesions by 4 dpi. TNVA, OMMV and PoNV developed chlorotic spots at about 3 dpi and these developed into bright chlorotic spots with necrosis 6-7 dpi. The resulting final symptoms were similar for all viruses.

Ch. quinoa can be used to distinguish PoNV from related viruses. TNVD and OMMV showed symptoms of chlorosis after 3 dpi, TNVA 4 dpi, but PoNV took 6-7 dpi before visible small chlorotic spots were observed (Fig. 3). For all viruses the chlorotic symptoms became necrotic and the leaves died. The distinction between PoNV and related viruses was striking but only visible in the short term (first week after inoculation). None of the *Chenopodium* spp produced systemic infections with any virus and the growth of all inoculated plants developed in a manner similar to the uninfected controls.

Nicotiana spp

N. benthamiana inoculated leaves show symptoms with all four viruses at 2 dpi, with systemic symptoms produced for all viruses. Differences were observed between viruses in the severity of systemic symptoms. TNVD produced the most severe symptoms, with the plant eventually dying. PoNV systemic symptoms were the least severe with the plant continuing to grow but with the new leaves showing puckering and mosaic symptoms (Fig. 4).

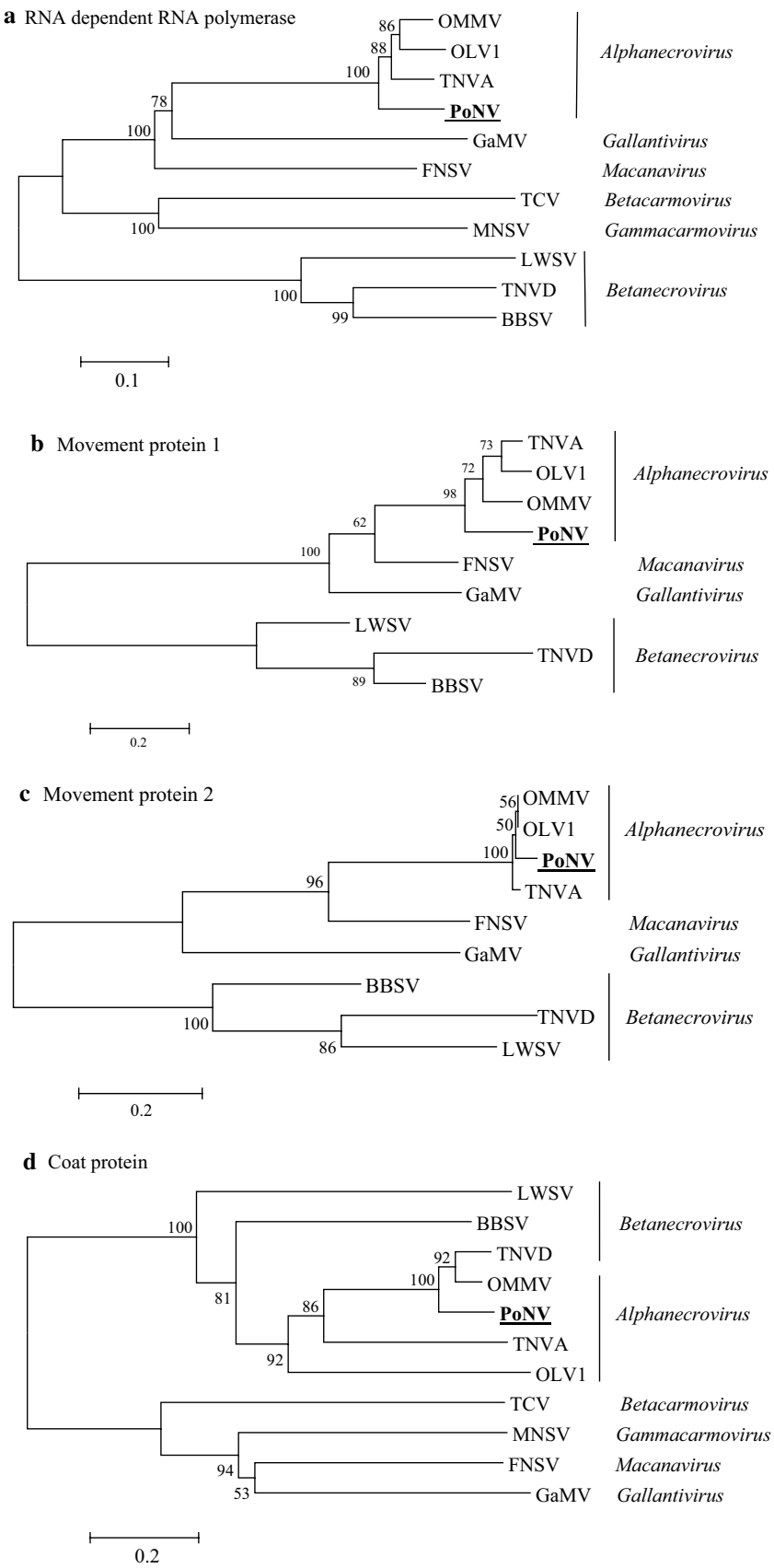

Fig. 2 Phylogenetic trees showing the relationship of PoNV encoded proteins with those from related viruses representing the family *Tombusviridae*. Trees were generated for the RNA dependent RNA polymerases (2a), movement proteins 1 and 2 (2b, 2c) and coat proteins (2d). PoNV (KP901095), OLV1 (X85989), OMMV (AY616760), TNVA (M33002), BBSV (AF452884), LWSV (X94560), TNVD (U62546), TCV (M22445), GaMV (Y13463), FNSV (FJ768020) and MNSV (M29671)

Table 3 Development of symptoms on *Chenopodium* and *Nicotiana* species by PoNV-QV323, OMMV-QV332, TNVA-QV322 and TNVD-QV330

Indicator plant	Virus	Inoculated leaves				Systemic
		2 dpi	3 dpi	4 dpi	7 dpi	
Ch. amaranticolor	PoNV	ns	ns	CS-m	CS	ns
	OMMV	ns	C	C, N	C, N	ns
	TNVA	ns	C	C, N	C, N	ns
	TNVD	C	CS	C, N	N, PT	ns
Ch. murale	PoNV	ns	CS-m	CS	CS, NS	ns
	OMMV	C	CS	CS	N, PT	ns
	TNVA	ns	CS	CS	NS	ns
	TNVD	CP	NP-s	N	N, PT	ns
Ch. quinoa	PoNV	ns	ns	CS-m	CS	ns
	OMMV	ns	CS	C, N	N, PT	ns
	TNVA	ns	C-m	C, N	N, PT	ns
	TNVD	C	CS	C, N	N	ns
N. benthamiana	PoNV	ns	NS	N, PT	N, PT	Sy-m
	OMMV	NP-m	NS	N, PT	N, PT	Sy
	TNVA	NP-m	NS	N, PT	N, PT	Sy
	TNVD	NP-m	NS	N, PT	N, PT	Sy
N. bigelovii	PoNV	ns	CS	N, PT	N, PT	ns
	OMMV	NP-s	N	N, PT	N, PT	ns
	TNVA	NP	N	N, PT	N, PT	ns
	TNVD	NP	N	N, PT	N, PT	ns
N. clevlandii	PoNV	NP	N	N, PT	N, PT	ns
	OMMV	NP-s	N	N, PT	N, PT	ns
	TNVA	NP-s	N	N, PT	N, PT	Sy
	TNVD	NP	N	N, PT	N, PT	ns
N. debneyi	PoNV	ns	CP-m, NP-m	CP, NP	CP, NS-f	ns
	OMMV	NP	N	N, PT	N, PT	ns
	TNVA	NP	N	N, PT	N, PT	ns
	TNVD	NP	N	N, PT	N, PT	ns
N. occidentalis-P1	PoNV	ns	N-m	N, PT	N, PT	ns
	OMMV	NP-s	N	N, PT	N, PT	ns
	TNVA	NP-s	N	N, PT	N, PT	ns
	TNVD	NP	N	N, PT	N, PT	ns
N. tabacum cv. White Burley	PoNV	ns	NS-m	CS-m, NS-m	CS, N-m	ns
	OMMV	NP-m	NP	N, PT	N, PT	ns
	TNVA	ns	NP	N, PT	N, PT	ns
	TNVD	ns	NP-m	C-m, N-m	C, N-m	ns

Symptoms on inoculated leaves were recorded at days post inoculation (dpi) and systemic symptoms were recorded. Symptom abbreviations are ns (no symptoms), C (chlorosis), CP (chlorotic pitting), CS (chlorotic spots), N (necrosis), NP (necrotic pitting), NS (necrotic spots), PT (paper thin necrosis), Sy (systemic) and severity abbreviations are -f (few), -m (mild), -s (severe)

N. bigelovii inoculated leaves showed symptoms of necrotic pitting at 2 dpi with TNVA, TNVD and OMMV. PoNV developed symptoms slower, with the first symptom of chlorotic spots at 3 dpi. At 7 dpi symptoms on inoculated leaves were equally severe with all viruses. Systemic infection was limited to one leaf above the inoculated leaf and this was seen with all the viruses in the form of some necrotic patches to the leaf. Thereafter, all plants continued to grow in a manner similar to the uninfected control. Asymptomatic leaves near the top of all plants were tested using RT-PCR and were negative for virus.

N. clevlandii showed similar symptoms that developed at the same time on inoculated leaves for all the viruses. Both TNVA isolates showed systemic symptoms resulting in stunted and deformed plants. This experiment was repeated a number of times and on one occasion one of the inoculated

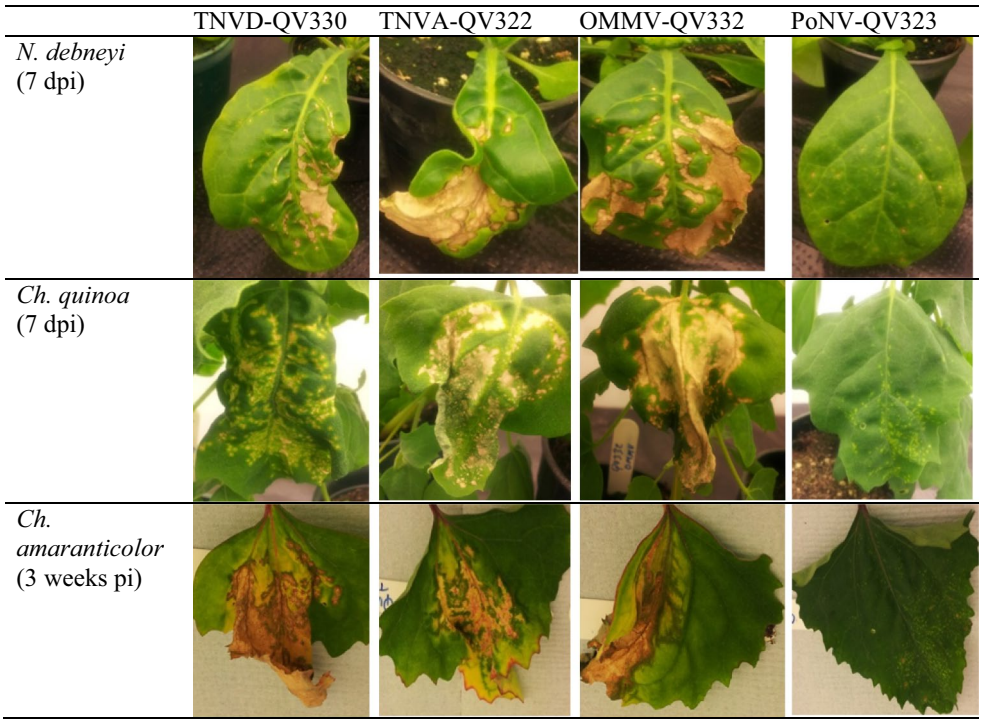

Fig. 3 Symptom development on the inoculated leaves of *Nicotiana debneyi*, *Chenopodium quinoa* and *Ch. amaranticolor* by PoNV and related viruses, at 7 days, 7 days and 3 weeks post inoculation respectively. Tobacco necrosis virus D (TNVD), tobacco necrosis virus A (TNVA), olive mild mosaic virus (OMMV) and potato necrosis virus (PoNV)

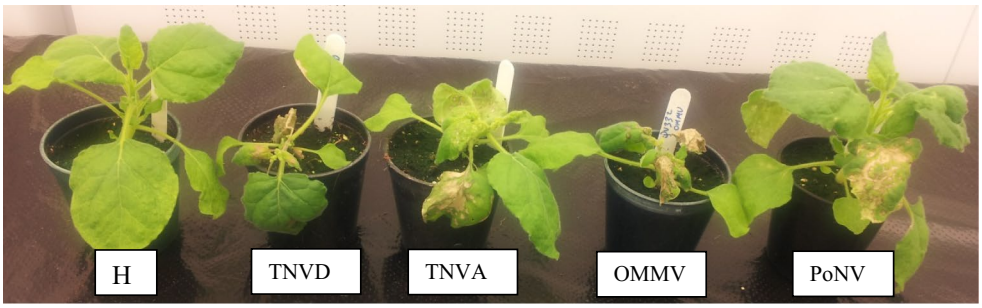

Fig. 4 *Nicotiana benthamiana* plants showing symptoms of infection by PoNV and related viruses at 7 days post inoculation. Uninfected (H), tobacco necrosis virus D (TNVD), tobacco necrosis virus A (TNVA), olive mild mosaic virus (OMMV), potato necrosis virus (PoNV)

PoNV plants also became systemically infected. In addition, symptoms on the leaf above the inoculated one were sometimes observed with OMMV and TNVD plants.

N. debneyi can be used to distinguish PoNV from related viruses. Inoculated leaves with TNVA, TNVD and OMMV developed pitting (small necrotic spots) 2-3 dpi, these quickly became large necrotic spots on the inoculated leaves. PoNV produced less severe symptoms that developed later; small chlorotic spots with some necrosis were visible at 4 dpi. Repeated inoculations showed that some PoNV inoculated leaves developed more severe symptoms by 7 dpi than those in Fig. 3 but none gave symptoms as severe as those produced with the other viruses.

N. occidentalis inoculated leaves showed symptoms of necrosis with TNVA, TNVD and OMMV at 2 dpi and PoNV at 3 dpi, although by 4 dpi the symptoms of necrosis and paper thin leaves were the same for all viruses.

N. tabacum cv. White Burley inoculated leaves produced severe symptoms of necrosis and paper thin leaves with OMMV and TNVA, whereas TNVD and PoNV produced mild chlorotic and necrotic symptoms.

DAS-ELISA

ELISA was used to test the specificity of TNVA and TNVD antisera to sap extracts from virus infected *N. benthamiana*. The OD after 2 h for each virus was as follows:

TNVA antisera detected TNVA-QV322 (OD_{405} 2.63) and PoNV-QV323 (OD_{405} 0.89). It did not detect TNVD-QV330

(OD_{405} 0.12) or OMMV-QV332 (OD_{405} 0.15) that were equivalent to the negative control (OD_{405} 0.12).

TNVD antisera detected TNVD-QV330 (OD_{405} 2.59), OMMV-QV332 (OD_{405} > 3.0) and PoNV-QV323 (OD_{405} 0.81) but not TNVA-QV322 (OD_{405} 0.10) that was equivalent to the negative control (OD_{405} 0.09). Readings after 24 h did not alter the conclusions.

Discussion

The complete nucleotide sequence has been deduced for a novel virus found in potato (cv. Nadine) grown in the UK. The phylogenetic analysis (Figs. 2), based on four encoded proteins, consistently grouped this virus with OMMV, OLV1 and TNVA (alphanecroviruses), with the ORFs in the same genome order as OLV1 and OMMV. OMMV was the closest relative in both conserved sequence motifs and identity (Table 2). The alphanecroviruses have a few nucleotides that separate the ORFs of MP1 and MP2, but PoNV differs with the last adenine base of the MP1 stop codon being the first adenine base of the MP2 start codon (Fig. 1, nt 2442).

The present ICTV molecular criteria for a new species within the genus *Alphanecrovirus* [20] is based on the differences found between the three known members TNVA, OMMV and OLV1, that is less than 93% and 55% aa sequence identity for the RdRp and CPs respectively. PoNV meets the present criteria for the RdRp protein, with the highest identity found with OMMV (88%), but fails for the CP where it shares 76% identity with OMMV. The present 55% CP criteria for an alphanecrovirus would also fail to distinguish OMMV from TNVD. The CP sequences for isolates of OLV1, TNVD and OMMV have been studied and found to be highly conserved within their species, with minor differences attributed to adaption to new host plants rather than geographical location or year of collection [27, 28]. In addition, the revised criterion for carmovirus demarcation only uses phylogenetic trees based on complete RdRps [22]. Therefore, demarcation criteria based on the CP is not appropriate for these viruses.

Host range and symptoms

Symptoms of necrovirids on indicator plants have been reported previously [1, 7]. They are produced quickly on inoculated leaves and are predominantly necrotic lesions that fail to go systemic in most plant species. An isolate of OLV1 was not available but host range and symptoms have been recorded [1]. No sequence was available for Chenopodium necrosis virus (ChNV), a virus first isolated from soil sample and subsequently from water in the UK [25]. This virus has physical properties consistent with a necrovirid and is serologically related to TNV-D. ChNV differs from other necrovirids by producing both local and systemic infections on *Ch. amaranticolor* and *Ch. quinoa* but not infecting *N. tabacum* cv. White Burley [25].

This study compared the symptoms produced and their time to develop with PoNV, TNVA, OMMV and TNVD on nine different *Chenopodium* and *Nicotiana* spp. In general, symptoms developed slower and were less severe with PoNV than related viruses. No other virus produced symptoms slower than PoNV, and five plant species had significantly slower development: *Ch. amaranticolor, Ch. quinoa, N. bigelovii, N. debneyi and N. occidentalis.* Milder symptoms were observed with PoNV compared to the other viruses on *Ch. amaranticolor, Ch. quinoa* and *N. debneyi,* with milder systemic symptoms produced on *N. benthamiana*. *Ch. amaranticolor* showed the most striking differences; whilst the related viruses (OMMV, TNVA and TNVD) showed symptoms 2-3 dpi, that turned necrotic, PoNV showed only faint chlorotic spots after a week that did not turn necrotic. Both *N. bigelovii* and *N. clevelandii* were observed to have partial-systemic infections with some viruses. This may be a true result or the effect of inoculum splatter from the inoculation procedure.

In this study PoNV was not mechanically transmitted to potato. Mechanical inoculation of potato by alphanecroviruses is known to be difficult [1]. It may not be possible for the virus to be transmitted in this manner or susceptibility to inoculum may require certain conditions. Although, members of this genus have been shown to be transmitted in soil by the fungus *Olpidium brassicae* [26] and therefore it is likely that PoNV would also transmit in this manner.

Recombination and detection

The synthesis of tombusvirid proteins involves a non-typical mechanism resulting from the uncapped and non-polyadenylated nature of their genomes. This mechanism has been a contributing factor in the recombinant and genetic rearrangement seen with members of this family, that have been studied for their tendency to evolve through recombination [2, 21, 30]. OMMV was originally identified as a strain of TNVD [5]. Further investigations using the complete genome sequence suggested OMMV to be an apparent recombinant between TNVD and OLV1 [6]. Whilst a clear distinction can be made between alpha and betanecroviruses with regard to their RdRp and MPs, the CP sequence alignment gave a less defined relationship. This study suggests the CP of TNVD has a closer relationship with the alphanecroviruses (Fig. 2d), implying TNVD is the recombinant. However, it is possible these viruses have undergone multiple recombination events during their evolution, making it difficult to conclude which viruses are ancestral and which recombinant. A clearer picture may emerge as more members of this family are discovered but

with such mixed origins, these viruses are unlikely to fit neatly into any taxonomic classification scheme.

There are two viruses with the name 'tobacco necrosis virus'. Once thought to be different isolates of the same virus they were named TNVA and TNVD. Although it has been some years since both were fully sequenced and shown to be distinct viruses, confusion continues in the literature, particularly in the non-scientific sector, with the name TNV being used for both viruses. The TNVs are not considered to be a serious threat to potato production and only TNVD has been reported in potato [16]. Due to the lack of published information on these viruses in potato, most diagnostic laboratories employ bioassays that would detect both. However, ELISA and molecular assays may also be used and the discovery of this new virus in potato raises issues for these methods. The CP of PoNV shares high identity with TNVD (78%) and an ELISA with TNVD antisera would be expected to react and has been shown to in this study. On the other hand, TNVA shares a lower degree of identity for the CP (49%) and although the TNVA antisera used in this study did detect PoNV, it is likely other antisera to TNVA may not. Molecular assays are designed to specific regions of the genome and this virus would likely be identified as either TNVA or TNVD depending on whether the assay was designed to the RdRp or CP regions. In addition, an assay designed to TNVA or TNVD in a region of low identity with PoNV would likely fail to detect PoNV. Misidentification of viruses in this family has come from single gene sequencing and serology [11]. For identification purposes tombusvirid members should not be identified with single gene analysis [23], and complete genomes are considered necessary before precise relatedness can be confirmed [2].

PoNV meets the molecular criteria for the RdRp protein to be recognised as an isolate of a separate species in the genus *Alphanecrovirus* [20]. PoNV exhibits a range of distinct symptoms with different indicator plants, that suggest a less aggressive virus than other members of the genus. The virus has been given the provisional name of potato necrosis virus (PoNV).

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Compliance with ethical standards

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

Research involving human participation and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

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