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Expansion of the plant-parasitic nematode RNA viruses: Unprecedented diversity, intron-bearing viruses, and cross-kingdom evolutionary links



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Plant-parasitic nematodes (PPNs) pose a significant threat to global crop production, yet their associated viral diversity remains poorly characterized, limiting potential virus-mediated biocontrol strategies. In this study, we investigated PPN-associated viruses using both virome data obtained from ten field populations of potato rot nematode (*Ditylenchus destructor*) collected in Lulong County (Qinhuangdao city, China), a major sweet potato-producing region, along with 536 publicly available transcriptome datasets from the Sequence Read Archive (SRA) database, collectively encompassing twenty-five PPN species. We identified 94 PPN-associated viruses, representing a 7.9-fold increase over prior records. These viruses span eighteen established families and six unclassified viral groups, including the first discovery of orthomyxo-like viruses, Jingmen viruses, and ormycoviruses in PPNs or nematodes, expanding the possible host ranges of these viral groups. Notably, a clade of yue-like viruses harbored up to 10 introns, surpassing 2–3 introns that were only observed in orthomyxoviruses and certain members of the *Mononegavirales*. Furthermore, we identified two larger nematode-associated bunyaviruses with the L segments exceeding 12,000 bp, which appear to have acquired a putative cysteine proteinase gene potentially originating from their nematode hosts (possibly *Pristionchus* spp.). Our findings reveal that natural PPN populations could host an unexpectedly high diversity of RNA viruses, higher than previously recognized. Exploring these viruses provides novel insights into viral evolution and establishes a foundation for utilizing viruses as a potential method for controlling PPN diseases.

Background

Nematodes, representing one of the most abundant and functionally diverse animal groups on Earth, serve as critical drivers of ecosystem processes^{1,2}. Among them, plant-parasitic nematodes (PPNs), encompassing over 4100 identified species, infest nearly all major crops across diverse agroecosystems, causing extensive damage and yield loss, and posing a severe threat to global food security^{3–5}. PPNs typically penetrate plant cell walls using a

specialized stylet, injecting saliva and secreting effectors that disrupt plant immunity and manipulate plant physiological metabolism to facilitate nutrient acquisition^{6–8}. Furthermore, their interactions with other organisms or phytopathogens often lead to disease complexes, complicating disease management efforts^{9,10}. Although fungal and bacterial biocontrol agents have demonstrated potential as sustainable tools for managing nematode diseases, the application of virus-based strategies against PPNs

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remains underexplored compared to their established application in controlling other plant pathogens and pests, including fungi, bacteria, and insects^{6,11,12}.

The ecological interplay between nematodes and their associated viruses remains an underexplored frontier. Recent advances have gradually uncovered nematode-associated viruses, beginning with the identification of indigenous viruses in *Caenorhabditis elegans* (e.g., Orsay virus) and *Caenorhabditis briggsae* (e.g., Santeuil virus, Le Blanc virus, and Melnik virus), which established these organisms as valuable systems for studying virus-host interactions^{13–16}. These discoveries support the hypothesis that nematode-infecting viruses may be more prevalent than previously recognized. Large-scale invertebrate virus investigations have further identified RNA virus infections in diverse nematodes, including snake-associated, mosquito-infecting, and mouse liver tissue-parasitizing species^{17,18}.

The limited characterization of PPN-associated viral diversity represents one of the major constraints on the development of virus-based strategies against PPNs. To date, to the best of our knowledge, only 12 RNA viruses classified into six orders/families, including *Phenuiviridae*, *Rhabdoviridae*, *Flaviviridae*, *Nyamiviridae*, *Picornavirales*, and unclassified bunyaviruses, have been identified in four PPNs (soybean cyst nematode, sugar beet cyst nematode, potato cyst nematode, and root lesion nematode) (Supplementary Table 1)^{19–24}. Additionally, some PPNs may serve as vectors for plant viruses, although such viruses typically do not replicate within nematodes^{25,26}. Compared to the viral diversity of soil-free nematodes or animal-parasitic nematodes^{27,28}, viruses of PPNs remain largely unexplored.

The discovery of viruses in nematodes could lead to new understandings and improved management for nematode-related diseases. In this study, we collected potato rot nematodes (PRNs) from infested sweet potatoes and conducted metatranscriptomic sequencing to investigate PRN-associated viruses. Furthermore, we analyzed transcriptome data from 25 PPN species across 536 public SRA runs to systematically uncover PPN-associated viral sequences. Our results revealed 94 new PPN or PPN-associated viruses within eighteen established families and six unclassified viral groups, which greatly highlight the PPN viral diversity and provide key insights into their evolution.

Results

Overview of PPN virome

To explore viral diversity in PPNs, we analyzed 536 publicly available RNA-seq SRA datasets (up to March 30, 2022), representing 25 PPN species. Additionally, we investigated the virome of ten field populations of potato rot nematode (PRN) collected from Lulong County, Qinghuangdao City, Hebei, China (Supplementary Fig. 1). Total RNA was extracted from the isolated PRNs and sequenced using an rRNA-depletion approach. Through the integration of viral sequences derived from both field-collected PRNs and public SRA datasets, we identified 94 RNA viruses with likely infection with PPNs (Table 1). Furthermore, 85 RNA viruses were excluded as potential contaminants based on the following criteria (Supplementary Table 2): (1) sequence similarity to known viruses from non-nematode hosts, such as fungi or oomycetes, and (2) co-occurrence of sequencing reads from these putative hosts within the same SRA run. These viruses, probably not hosted by PPNs, are generally designated as “PPN-associated virus” in this study as shown in Supplementary Table 2. A virus was classified as “PPN-infected” if it satisfied one of the following conditions: (1) sequence similarity to viruses of unknown host origin and a low ratio of non-nematode reads; or (2) phylogenetic clustering with previously reported or newly identified nematode viruses. The remaining viruses with uncertain hosts are also designated as “PPN-associated virus” in this study to distinguish them from confirmed “PPN viruses” (Table 1). Although experimental validation is required for definitive confirmation, such inferences are critical for viromic studies. Taxonomic summaries of the predominant organisms in some key SRA runs associated with these viruses are provided in the Supplementary Note 1.

Among 94 PPN-associated viruses, 34 are negative-sense single-stranded ((-)ss) RNA viruses, classified within *Bunyaviricetes* ($n = 14$),

Rhabdoviridae ($n = 5$), *Nyamiviridae* ($n = 5$), *Goujianvirales* ($n = 6$), *Qinviridae* ($n = 2$), and *Orthomyxoviridae* ($n = 2$). Furthermore, 57 (+)ssRNA viruses are phylogenetically related to members of *Endornaviridae* ($n = 3$), *Nodaviridae* ($n = 2$), unclassified *Martellivirales* ($n = 3$), *Tymoviridae* ($n = 1$), *Tombusviridae* ($n = 2$), *Flaviviridae* ($n = 8$), *Picornavirales* ($n = 23$), *Astroviridae* ($n = 3$), and *Bormyovirales* ($n = 12$). Additionally, three dsRNA viruses were identified, belonging to *Birnaviridae* ($n = 1$) and *Amalgaviridae* ($n = 2$) (Table 1 and Fig. 1A). Although two species, *Bursaphelenchus xylophilus* (126 SRA runs) and *Globodera pallida* (92 SRA runs), accounted for 41% of the total dataset (218/536), relatively low RNA virus diversity was detected (Fig. 1B and Supplementary Table 3). This may be due to redundancy in the downloaded SRA runs, which stems from the widespread use of certain strains in experimental design. The nematodes *Ditylenchus destructor* and *Heterodera glycines* displayed high viral diversity (Fig. 1B). Most viruses detected in *D. destructor* (25/26) originated from the PRN field populations. Over 83% of identified nematode-associated viral genome segments (131/157) exhibited low amino acid (aa) identity (<50%) with previously reported viruses (Table 1, Supplementary Fig. 2). These findings imply that there is a yet largely unexplored viral diversity within field PPN populations. Furthermore, our analyses revealed that potato cyst nematode picorna-like virus 1 (PCNPV1) and potato cyst nematode rhabdovirus 1 (PCNRHV1) are among the most abundant viruses across all analyzed SRA runs (Fig. 1C), indicating that the PCN strain commonly used in general research may be infected with these viruses.

Novel negative-sense single-stranded RNA viruses

(i) Mononegavirales. (a) Rhabdoviridae. We identified five rhabdoviruses, including three newly identified ones—potato rot nematode rhabdovirus 1 (PRNRHV1), soybean cyst nematode rhabdovirus 1 (SCNRHV1), and soybean cyst nematode rhabdovirus 2 (SCNRHV2)—as well as two previously reported rhabdoviruses—soybean cyst nematode associated northern cereal mosaic virus rhabdovirus (SCNNCMV, HM849039.2) and potato cyst nematode rhabdovirus 1 (PCNRHV1, OP903920.1). The complete genome of PRNRHV1 is 13,233 nt in length and exhibits terminal complementarity at the 3' and 5' ends. It contains five non-overlapping open reading frames (ORF I–V), encoding the five canonical rhabdovirus proteins: nucleoprotein (N), phosphoprotein (P), membrane protein (M), glycoprotein (G), and polymerase (L) (Fig. 2). The intergenic regions of PRNRHV1 feature conserved transcription start (CAAAGACAACAA) and stop (UUAGAAAAAA) signals, consistent with those of canonical rhabdoviruses²⁹. For SCNRHV1 and SCNRHV2, only partial genomes were assembled, with lengths of approximately 11,472 nt and 7640 nt, respectively, excluding predicted gap regions (Fig. 2). The L proteins of PRNRHV1, SCNRHV1, and SCNRHV2 share the highest similarity to PCNRHV1, exhibiting 41%, 45%, and 56% aa identities, respectively (Table 1). The three newly identified nematode rhabdoviruses cluster with previously reported SCNNCMV and PCNRHV1, forming a clade within *Rhabdoviridae* family with a UFBoot support value greater than 90%. The existence of this clade suggests significant host specificity among nematode-associated rhabdoviruses (Fig. 2).

(b) Nyamiviridae. We identified five viruses belonging to *Nyamiviridae* from publicly available SRA data of PPNs, including two newly identified ones—soybean cyst nematode nyami-like virus 2 (SCNNV2) and stem and bulb nematode nyami-like virus 1 (SBNNV1)—as well as three previously reported ones—soybean cyst nematode midway virus (SCNMV1, HM849038)²⁰ and soybean cyst nematode nyami-like viruses (SCNNV, MG550266 and MG550268)²³. Notably, we found that the RdRP encoded by two versions of SCNNV (MG550266 and MG550268) in GenBank share about 74% aa identity, suggesting they are distinct virus species. Given that we assembled a longer version of SCNNV (MG550268, 1815 nt) from a sugar beet cyst nematode library (SRR16675965), we renamed this partial sequence as sugar beet cyst nematode nyami-like virus 1 (SBCNNV1) in this study. Phylogenetic

Table 1 | Summary of identified viruses that probably infected or are associated with PPNs

Genome type	Classification	Virus	Abbreviation	Accession	Genome (nt)	Host	Representative SRA	Best hit (BLASTp)	Query coverage	Identity	E-value
(-)ssRNA	Unclassified bunyaviruses	Potato rot nematode bunyavirus 1	PRNBVY1-L	PV640383	12,325	<i>Ditylenchus destructor</i>	SRR28892574	WWV90583 MAG: RNA polymerase, partial [Riboviria sp.]	56%	30%	0
	Unclassified bunyaviruses	Potato rot nematode bunyavirus 2	PRNBVY2-L	PV640384	12,018	<i>Ditylenchus destructor</i>	SRR28892574	WWV90583 MAG: RNA polymerase, partial [Riboviria sp.]	59%	29%	0
Unclassified bunyaviruses	Soybean cyst nematode bunya-like virus	SCNBLV-L	BK071874	9452	<i>Heterodera glycines</i>	SRR6269844	SRR6269844	AVK42877 RNA-dependent RNA polymerase [Soybean cyst nematode bunya-like virus]	100%	100%	0
	Soybean cyst nematode associated bunya-like virus 2	SCNBLV2-L	BK071875	4800	Unknown	SRR9647096	SRR9647096	QJD26146 polymerase [Schistocephalus solidus bunya-like virus]	73%	37%	0
Unclassified bunyaviruses	Potato rot nematode bunyavirus 3	PRNBVY3-L	PV640385	6809	<i>Ditylenchus destructor</i>	SRR28892574	SRR28892574	QJD26146 polymerase [Schistocephalus solidus bunya-like virus]	85%	31%	0
Unclassified bunyaviruses	Cereal cyst nematode associated bunya-like virus 1	CCNBVY1-L	BK071886	4809	Unknown	SRR5942326	SRR5942326	QJD26146 polymerase [Schistocephalus solidus bunya-like virus]	96%	32%	0
<i>Phenuiviridae</i>	Potato rot nematode bunyavirus 5	PRNBVY5-L	PV640391	7100	<i>Ditylenchus destructor</i>	SRR28892574	SRR28892574	WPV71150 MAG: RNA-dependent RNA polymerase [lps phenui-like virus 2]	90%	32%	0
<i>Phenuiviridae</i>	Potato rot nematode bunyavirus 4	PRNBVY4-L	PV640386	7101	<i>Ditylenchus destructor</i>	SRR28892574	SRR28892574	WPV71150 MAG: RNA-dependent RNA polymerase [lps phenui-like virus 2]	87%	32%	0
<i>Phenuiviridae</i>	Potato rot nematode bunyavirus 6	PRNBVY6-L	PV640395	7456	<i>Ditylenchus destructor</i>	SRR28892574	SRR28892574	WPV62171 MAG: RNA-dependent RNA polymerase [Wenzhou rodent phenuivirus 1]	85%	35%	0
<i>Phenuiviridae</i>	Potato rot nematode associated bunyavirus 7	PRNBVY7-L	PV640397	2393	Unknown	SRR28892574	SRR28892574	DBA56479 TPA_asm: RNA-dependent RNA polymerase [Coptotermes formosanus phenuivirus 1]	86%	31%	1.02E-94
<i>Phenuiviridae</i>	Potato rot nematode associated bunyavirus 8	PRNBVY8-L	PV640398	2473	Unknown	SRR28892574	SRR28892574	UYL95518 RNA-dependent RNA polymerase [Tonghua tick virus 1]	85%	25%	2E-37
<i>Phenuiviridae</i>	Soybean cyst nematode associated rice stripe virus	SCNRSV-L	BK071878	8839	<i>Heterodera glycines</i>	SRR13711083	SRR13711083	AEF56735 RNA dependent RNA polymerase, partial [Soybean cyst nematode associated rice stripe virus]	73%	100%	0
<i>Phenuiviridae</i>	Soybean cyst nematode associated rice stripe virus	SCNRSV-S	BK071879	1175	<i>Heterodera glycines</i>	SRR13711083	SRR13711083	AAP06149 S_ICHGCO4696 protein, partial [Schistosoma japonicum]	34%	30%	0.004
<i>Phenuiviridae</i>	Soybean cyst nematode associated Uukuniemi virus	SCNUUKV-L	BK071880	6630	<i>Heterodera glycines</i>	SRR6269844	SRR6269844	AEF56734 RNA dependent RNA polymerase, partial [Soybean cyst nematode associated Uukuniemi virus]	100%	98%	0
<i>Phenuiviridae</i>	Soybean cyst nematode associated Uukuniemi virus	SCNUUKV-M	BK071881	3372	<i>Heterodera glycines</i>	SRR6269844	SRR6269844	QC084580 glycoprotein, partial [Austropotamobius brown spot virus]	75%	25%	1.92E-57
<i>Phenuiviridae</i>	Soybean cyst nematode associated Uukuniemi virus	SCNUUKV-S	BK071882	1469	<i>Heterodera glycines</i>	SRR6269844	SRR6269844	YP_010086152 nucleoprotein [Rukutama virus]	74%	33%	1.28E-23
<i>Phenuiviridae</i>	Soybean cyst nematode associated bunya-like virus 3	SCNBLV3-L	BK071876	4545	Unknown	SRR6230579	SRR6230579	AQW38600 RNA-dependent RNA polymerase [Sugar beet cyst nematode virus 2]	95%	33%	0

Table 1 (continued) | Summary of identified viruses that probably infected or are associated with PPNs

Genome type	Classification	Virus	Abbreviation	Accession	Genome (nt)	Host	Representative SRA	Best hit (BLASTp)	Query coverage	Identity	E-value
Phenuiviridae		Soybean cyst nematode associated bunya-like virus 3	SCNBLV3-S	BK071877	1769	Unknown	SRR6230579	QPN53432 N protein [Mourilyan virus]	80%	23%	1.2E-11
		Potato cyst nematode rhabdovirus 1	PCNRHV1	BK071890	13,675	<i>Globodera pallida</i>	ERR202422	WAK29061 RNA dependent RNA polymerase [Potato cyst nematode rhabdovirus]	100%	100%	0
Rhabdoviridae		Soybean cyst nematode rhabdovirus 1	SCNRHV1	BK071888	11,472	<i>Heterodera glycines</i>	SRR9647098	WAK29061 RNA dependent RNA polymerase [Potato cyst nematode rhabdovirus]	99%	45%	0
Rhabdoviridae		Soybean cyst nematode rhabdovirus 2	SCNRHV2	BK071889	7640	<i>Heterodera glycines</i>	SRR9647098	WAK29061 RNA dependent RNA polymerase [Potato cyst nematode rhabdovirus]	99%	57%	3.59E-176
Rhabdoviridae		Potato rot nematode rhabdovirus 1	PRNRHV1	PV640399	13,218	<i>Ditylenchus destructor</i>	SRR28892574	WAK29061 RNA dependent RNA polymerase [Potato cyst nematode rhabdovirus]	99%	41%	0
Rhabdoviridae		Soybean cyst nematode associated northern cereal mosaic virus	SCNNCMV	BK071887	12,737	<i>Heterodera glycines</i>	SRR6232817	AEF56733 putative RNA-dependent RNA polymerase [Soybean cyst nematode associated northern cereal mosaic virus]	100%	100%	0
Nyamiviridae		Sugar beet cyst nematode nyami-like virus 1	SBCNNV1	BK071894	12,400	<i>Heterodera schachtii</i>	SRR16675965	AVK42876 RNA dependent RNA polymerase, partial [Soybean cyst nematode nyami-like virus]	30%	99%	0
Nyamiviridae		Socovirus heteroderae	SCNMiDV1	BK071893	11,476	<i>Globodera pallida</i>	SRR6269845	YP_009052467 putative RNA dependent RNA polymerase [Socovirus heteroderae]	100%	99%	0
Nyamiviridae		Soybean cyst nematode nyami-like virus 1	SCNNV1	BK071891	11,690	<i>Heterodera glycines</i>	SRR6269844	AVK42865 RNA-dependent RNA polymerase [Soybean cyst nematode nyami-like virus]	100%	100%	0
Nyamiviridae		Soybean cyst nematode nyami-like virus 2	SCNNV2	BK071892	8690	<i>Heterodera glycines</i>	SRR9647098	AVK42865 RNA-dependent RNA polymerase [Soybean cyst nematode nyami-like virus]	100%	89%	0
Nyamiviridae		Stem and bulb nematode nyami-like virus 1	SBNNV1	BK071895	4613	<i>Ditylenchus dipsaci</i>	SRR8239758	AVK42865 RNA-dependent RNA polymerase [Soybean cyst nematode nyami-like virus]	94%	32%	0
Yueviridae		Potato rot nematode yue-like virus 1	PRNYV1-RNA1	PV640400	6411	<i>Ditylenchus destructor</i>	SRR28892574	YP_009337854 RNA-dependent RNA polymerase [Shahre yuevirus-like virus 1]	99%	29%	0
Yueviridae		Potato rot nematode yue-like virus 1	PRNYV1-RNA2	PV640401	1589	<i>Ditylenchus destructor</i>	SRR28892574	UNI74148 MAG: hypothetical protein [brine shrimp yue-like virus 2]	49%	36%	3.94E-27
Yueviridae		Potato rot nematode yue-like virus 2	PRNYV2-RNA1	PV640402	5932	<i>Ditylenchus destructor</i>	SRR28892574	QNS31060 putative RNA dependent RNA polymerase [Thrips tabaci associated yue-like virus 1]	87%	27%	5.41E-172
Yueviridae		Potato rot nematode yue-like virus 2	PRNYV2-RNA2	PV640403	3074	<i>Ditylenchus destructor</i>	SRR28892574	No significant hit			
Yueviridae		Potato rot nematode yue-like virus 2	PRNYV2-RNA3	PV640404	3043	<i>Ditylenchus destructor</i>	SRR28892574	No significant hit			
Unclassified Goujianvirales		Soybean cyst nematode yue-like virus 1a	SCNYV1a-RNA1	BK071896	6582	<i>Heterodera glycines</i>	SRR6232824	QNS31060 putative RNA dependent RNA polymerase [Thrips tabaci associated yue-like virus 1]	91%	26%	1.02E-173

Table 1 (continued) | Summary of identified viruses that probably infected or are associated with PPNs

Genome type	Classification	Virus	Abbreviation	Accession	Genome (nt)	Host	Representative SRA	Best hit (BLASTp)	Query coverage	Identity	E-value
Unclassified <i>Goujianvirales</i>		Soybean cyst nematode yue-like virus 1a	SCNYV1a-RNA2	BK071897	3225	<i>Heterodera glycines</i>	SRR6232824	No significant hit			
		Soybean cyst nematode yue-like virus 1b	SCNYV1b-RNA1	BK071898	6144	<i>Heterodera glycines</i>	SRR9647096	QNS31060 putative RNA dependent RNA polymerase [Thrips tabaci associated yue-like virus 1]	92%	26%	1.87E-174
		Soybean cyst nematode yue-like virus 1b	SCNYV1b-RNA2	BK071899	3210	<i>Heterodera glycines</i>	SRR9647096	No significant hit			
Unclassified <i>Goujianvirales</i>		Soybean cyst nematode yue-like virus 2	SCNYV2-RNA1	BK071900	6321	<i>Heterodera glycines</i>	SRR6230585	QNS31060 putative RNA dependent RNA polymerase [Thrips tabaci associated yue-like virus 1]	86%	27%	5.45E-162
		Soybean cyst nematode yue-like virus 2	SCNYV2-RNA2	BK071901	3285	<i>Heterodera glycines</i>	SRR6230585	No significant hit			
		Soybean cyst nematode yue-like virus 3	SCNYV3-RNA1	BK071902	6654	<i>Heterodera glycines</i>	SRR6230586	QNS31060 putative RNA dependent RNA polymerase [Thrips tabaci associated yue-like virus 1]	90%	26%	2.42E-160
Unclassified <i>Goujianvirales</i>		Soybean cyst nematode yue-like virus 3	SCNYV3-RNA2	BK071903	3219	<i>Heterodera glycines</i>	SRR6230586	No significant hit			
		Potato rot nematode qin-like virus 1	PRNQV1-RNA1	PV640405	6034	<i>Ditylenchus destructor</i>	SRR28892574	YP_009345119 RNA-dependent RNA polymerase [Xinzhou nematode virus 3]	70%	34%	0
		Potato rot nematode qin-like virus 1	PRNQV1-RNA2	PV640406	2425	<i>Ditylenchus destructor</i>	SRR28892574	YP_009345120 hypothetical protein [Xinzhou nematode virus 3]	64%	27%	3.46E-28
Qinviridae		Potato cyst nematode qin-like virus 1	PCNQV1-RNA1	BK071904	5787	<i>Globodera rostochiensis</i>	SRR16693885	YP_009345119 RNA-dependent RNA polymerase [Xinzhou nematode virus 3]	70%	34%	0
		Potato cyst nematode qin-like virus 1	PCNQV1-RNA2	BK071905	2261	<i>Globodera rostochiensis</i>	SRR16693885	WKY10110 hypothetical protein Q1695_002454 [Nippostrongylus brasiliensis]	85%	28%	1E-31
		Potato cyst nematode orthomyxo-like virus 1	PCNOV1-PB1	BK071906	2368	<i>Globodera rostochiensis</i>	ERR1173512	AVM87619 PB1, partial [Wenling orthomyxo-like virus 2]	82%	40%	3.5E-138
Orthomyxoviridae		Potato cyst nematode orthomyxo-like virus 1	PCNOV1-PB2	BK071907	2325	<i>Globodera rostochiensis</i>	ERR1173512	No significant hit			
		Potato cyst nematode orthomyxo-like virus 1	PCNOV1-GP	BK071908	2211	<i>Globodera rostochiensis</i>	ERR1173512	MCP4487896 DDE-type integrase/transposase/recombinase [Gammaproteobacteria bacterium]	70%	21%	2.26E-10
		Potato cyst nematode orthomyxo-like virus 1	PCNOV1-PA	BK071909	2140	<i>Globodera rostochiensis</i>	ERR1173512	QQQ34656 PA protein [Thogotovirus thogotoense]	47%	27%	1.7E-19
Orthomyxoviridae		Potato cyst nematode orthomyxo-like virus 1	PCNOV1-NP	BK071910	2004	<i>Globodera rostochiensis</i>	ERR1173512	No significant hit			
		Bursaphelenchus mucronatus orthomyxo-like virus 1	BMOV1-PB1	BK071911	2405	<i>Bursaphelenchus mucronatus</i>	SRR7062725	AVM87619 PB1, partial [Wenling orthomyxo-like virus 2]	81%	41%	1.04E-151
		Bursaphelenchus mucronatus orthomyxo-like virus 1	BMOV1-PB2	BK071912	2545	<i>Bursaphelenchus mucronatus</i>	SRR7062725	No significant hit			

Table 1 (continued) | Summary of identified viruses that probably infected or are associated with PPNs

Genome type	Classification	Virus	Abbreviation	Accession	Genome (nt)	Host	Representative SRA	Best hit (BLASTp)	Query coverage	Identity	E-value
	Orthomyxoviridae	Bursaphelenchus mucronatus orthomyxo-like virus 1	BMOV1-GP	BK071913	2263	<i>Bursaphelenchus mucronatus</i>	SRR7062725	MCP4487896 DDE-type integrase/transposase/recombinase [Gammaproteobacteria bacterium]	31%	24%	0.0001
		Bursaphelenchus mucronatus orthomyxo-like virus 1	BMOV1-PA	BK071914	2266	<i>Bursaphelenchus mucronatus</i>	SRR7062725	YP_010840384 PA polymerase subunit [Upolu virus]	44%	24%	0.00000001
(+) ssRNA	Orthomyxoviridae	Bursaphelenchus mucronatus orthomyxo-like virus 1	BMOV1-NP	BK071915	2368	<i>Bursaphelenchus mucronatus</i>	SRR7062725	No significant hit			
		Santeuil nodavirus	SANTV-RNA1	PV640420	3677	Unknown	SRR28892574	YP_004221742 RNA dependent RNA polymerase [Santeuil nodavirus]	99%	96%	0
Nodaviridae	Nodaviridae	Santeuil nodavirus	SANTV-RNA2	PV640421	2653	Unknown	SRR28892574	YP_004221743 capsid protein [Santeuil nodavirus]	100%	96%	0
		Lulong nodavirus	LLNDV-RNA1	PV640422	3452	Unknown	SRR28892574	UHM27590 MAG: RNA-dependent RNA polymerase, partial [Sanya nodavirus 1]	69%	32%	3.65E-105
Nodaviridae	Nodaviridae	Lulong nodavirus	LLNDV-RNA2	PV640423	2476	Unknown	SRR28892574	WKV33363 MAG: RNA-dependent RNA polymerase [Riboviria sp.]	47%	34%	1.76E-22
		Potato rot nematode endornavirus 1a	PRNEV1a	PV640407	14,218	<i>Ditylenchus destructor</i>	SRR28892574	CAH2618724.1 polyprotein [Krauss' spikemoss associated endorna-like virus]	78%	32%	0
Endornaviridae	Endornaviridae	Potato rot nematode endornavirus 1b	PRNEV1b	PV640408	14,105	<i>Ditylenchus destructor</i>	SRR28892574	CAH2618724.1 polyprotein [Krauss' spikemoss associated endorna-like virus]	78%	32%	0
		Potato rot nematode endornavirus 1c	PRNEV1c	PV640409	11,147	<i>Ditylenchus destructor</i>	SRR28892574	QUA12642.1 polyprotein, partial [Phytophthora cactorum alphaendornavirus 3]	74%	27%	5.33E-162
Unclassified Martellivirales	Unclassified Martellivirales	Soybean cyst nematode associated virga-like virus 1	SCNV1	BK071916	8997	<i>Heterodera glycines</i>	SRR9647096	YP_009333242 RdRP [Beihei charybdis crab virus 1]	56%	35%	9.17E-137
Unclassified Martellivirales	Unclassified Martellivirales	Sugar beet cyst nematode associated virga-like virus 1	SBCNV1	BK071918	5961	Unknown	SRR16675965	QWC36507 polyprotein [Bemisia tabaci bromo-like virus 1]	69%	38%	4.27E-78
Unclassified Martellivirales	Unclassified Martellivirales	Bursaphelenchus mucronatus associated virga-like virus 1	BMV1	BK071917	9651	Unknown	SRR7062723	UTQ50504.1 polyprotein [Plant associated tobamo-like virus 1]	27%	37%	3E-87
Tymoviridae	Tymoviridae	Potato cyst nematode associated tymovirus 1	PCNTV1	BK071919	8724	<i>Globodera pallida</i>	ERR202430	QQG34657 replicase, partial [Sinomenium acutum tymovirus 1]	78%	33%	1.53E-153
Tombusviridae	Tombusviridae	Potato cyst nematode associated tombus-like virus 1	PCNTomV1	BK071977	4223	Unknown	SRR1873823	USC30247 MAG: putative RNA dependent RNA polymerase, partial [Tombusviridae sp.]	49%	83%	0
Tombusviridae	Tombusviridae	Soybean cyst nematode associated tombus-like virus 1	SCNTomV1	BK071978	4300	Unknown	SRR6269844	WRQ65088 RNA-dependent RNA polymerase, partial [Tolivirales sp.]	70%	70%	0
Flaviviridae	Flaviviridae	Soybean cyst nematode virus 5	SCNV5	BK071920	18,835	<i>Heterodera glycines</i>	SRR9647098	YP_009028573 polyprotein [Soybean cyst nematode virus 5]	99%	94%	0
Flaviviridae	Flaviviridae	Cereal cyst nematode Jingmen virus 1	CCNJMV1-NS5	BK071921	3392	<i>Heterodera avenae</i>	SRR5942326	QPC92984 NS5 protein [Jingmen tick virus]	84%	32%	2.11E-124

Table 1 (continued) | Summary of identified viruses that probably infected or are associated with PPNs

Genome type	Classification	Virus	Abbreviation	Accession	Genome (nt)	Host	Representative SRA	Best hit (BLASTp)	Query coverage	Identity	E-value
Flaviviridae	Flaviviridae	Cereal cyst nematode Jingmen virus 1	CCNJMV1-NS3	BK071922	3006	<i>Heterodera avenae</i>	SRR5942326	QPG92986 NS3 protein [Jingmen tick virus]	68%	29%	5.2E-42
		Cereal cyst nematode Jingmen virus 1	CCNJMV1-GP1	BK071923	2636	<i>Heterodera avenae</i>	SRR5942326	No significant hit			
		Cereal cyst nematode Jingmen virus 1	CCNJMV1-GP2	BK071924	1962	<i>Heterodera avenae</i>	SRR5942326	No significant hit			
Flaviviridae	Flaviviridae	Cereal cyst nematode Jingmen virus 2	CCNJMV2-NS5	BK071925	3399	<i>Heterodera avenae</i>	SRR5942326	QPG92984 NS5 protein [Jingmen tick virus]	79%	34%	4.67E-123
		Cereal cyst nematode Jingmen virus 2	CCNJMV2-NS3	BK071926	3031	<i>Heterodera avenae</i>	SRR5942326	ACF19855 ANT-34 [Toxocara canis]	63%	28%	8.12E-38
		Cereal cyst nematode Jingmen virus 2	CCNJMV2-GP	BK071927	3115	<i>Heterodera avenae</i>	SRR5942326	No significant hit			
Flaviviridae	Flaviviridae	Cereal cyst nematode Jingmen virus 3	CCNJMV3-NS5	BK071928	3131	<i>Heterodera avenae</i>	SRR5942326	UXD80086 putative RdRP [Lasius neglectus virus 4]	87%	31%	3.72E-95
		Cereal cyst nematode Jingmen virus 3	CCNJMV3-NS3	BK071929	3048	<i>Heterodera avenae</i>	SRR5942326	UXD80087 hypothetical protein [Lasius neglectus virus 4]	76%	27%	4.26E-42
		Cereal cyst nematode Jingmen virus 3	CCNJMV3-GP	BK071930	2737	<i>Heterodera avenae</i>	SRR5942326	No significant hit			
Flaviviridae	Flaviviridae	Cereal cyst nematode Jingmen virus 4	CCNJMV4-NS5	BK071931	701	<i>Heterodera avenae</i>	SRR5942326	UYO80033 RNA-dependent RNA polymerase [Amarillovirales sp.]	98%	32%	2.94E-30
		Cereal cyst nematode Jingmen virus 4	CCNJMV4-NS3	BK071932	1788	<i>Heterodera avenae</i>	SRR5942326	UXD80087 hypothetical protein [Lasius neglectus virus 4]	76%	26%	1.93E-29
		Cereal cyst nematode Jingmen virus 4	CCNJMV4-GP	BK071933	3472	<i>Heterodera avenae</i>	SRR5942326	No significant hit			
Flaviviridae	Flaviviridae	Cereal cyst nematode Jingmen virus	CCNJMV-UnCP1	BK071934	1680	<i>Heterodera avenae</i>	SRR5942326	No significant hit			
		Cereal cyst nematode Jingmen virus	CCNJMV-UnCP2	BK071935	1629	<i>Heterodera avenae</i>	SRR5942326	No significant hit			
		Cereal cyst nematode Jingmen virus	CCNJMV-UnCP3	BK071936	3119	<i>Heterodera avenae</i>	SRR5942326	No significant hit			
Flaviviridae	Flaviviridae	Cereal cyst nematode Jingmen virus	CCNJMV-UnCP4	BK071937	1741	<i>Heterodera avenae</i>	SRR5942326	No significant hit			
		Cereal cyst nematode Jingmen virus	CCNJMV-UnCP5	BK071938	1630	<i>Heterodera avenae</i>	SRR5942326	No significant hit			
		Cereal cyst nematode Jingmen virus	CCNJMV-UnCP6	BK071939	1663	<i>Heterodera avenae</i>	SRR5942326	No significant hit			
Flaviviridae	Flaviviridae	Cereal cyst nematode Jingmen virus	CCNJMV-UnCP7	BK071940	1663	<i>Heterodera avenae</i>	SRR5942326	No significant hit			
		Potato cyst nematode Jingmen virus 1	PCNJMV1-NS5	BK071941	3405	<i>Globodera rostochiensis</i>	ERR1173512	QHD64751 NS5 [Plasmodium viticola lesion associated Jingman-like virus 1]	82%	32%	2.93E-128
		Potato cyst nematode Jingmen virus 1	PCNJMV1-NS3	BK071942	3097	<i>Globodera rostochiensis</i>	ERR1173512	ACF19855 ANT-34 [Toxocara canis]	62%	34%	2.68E-59
Flaviviridae	Flaviviridae	Potato cyst nematode Jingmen virus 1	PCNJMV1-GP	BK071943	3308	<i>Globodera rostochiensis</i>	ERR1173512	No significant hit			

Table 1 (continued) | Summary of identified viruses that probably infected or are associated with PPNs

Genome type	Classification	Virus	Abbreviation	Accession	Genome (nt)	Host	Representative SRA	Best hit (BLASTp)	Query coverage	Identity	E-value
Flaviviridae	Flaviviridae	Potato cyst nematode Jingmen virus 1	PCNJMV1-CP	BK071944	1560	<i>Globodera rostochiensis</i>	ERR1173512	No significant hit			
	Flaviviridae	Potato cyst nematode Jingmen virus 2	PCNJMV2-NS5	BK071945	3373	<i>Globodera rostochiensis</i>	ERR202480	QPG92984 NS5 protein [Jingmen tick virus]	83%	33%	1.19E-124
	Flaviviridae	Potato cyst nematode Jingmen virus 2	PCNJMV2-NS3	BK071946	3072	<i>Globodera rostochiensis</i>	ERR202480	ACF19855 ANT-34 [Toxocara canis]	61%	32%	2.17E-54
	Flaviviridae	Potato cyst nematode Jingmen virus 2	PCNJMV2-GP	BK071947	3013	<i>Globodera rostochiensis</i>	ERR202480	No significant hit			
Flaviviridae	Flaviviridae	Potato cyst nematode Jingmen virus 2	PCNJMV2-CP	BK071948	1686	<i>Globodera rostochiensis</i>	ERR202480	No significant hit			
	Flaviviridae	Sugar beet cyst nematode Jingmen virus 1	SBCNJMV1-NS5	BK071949	2655	<i>Heterodera schachtii</i>	SRR16675965	KAK6042405 flavivirus RNA-directed RNA polymerase [Cooperia oncophora]	69%	39%	3.66E-131
Flaviviridae	Flaviviridae	Sugar beet cyst nematode Jingmen virus 1	SBCNJMV1-NS3	BK071950	3201	<i>Heterodera schachtii</i>	SRR16675965	ACF19855 ANT-34 [Toxocara canis]	61%	31%	2E-54
Flaviviridae	Flaviviridae	Sugar beet cyst nematode Jingmen virus 1	SBCNJMV1-GP	BK071951	3225	<i>Heterodera schachtii</i>	SRR16675965	No significant hit			
Flaviviridae	Flaviviridae	Sugar beet cyst nematode Jingmen virus 1	SBCNJMV1-CP	BK071952	1670	<i>Heterodera schachtii</i>	SRR16675965	No significant hit			
Marnaviridae	Marnaviridae	Root-knot nematode associated marnavirus 1	RKNMV1	BK071953	9005	<i>Meloidogyne incognita</i>	SRR17756052	XCM67804 MAG; polyprotein [Picornavirales sp.]	97%	59%	0
Marnaviridae	Marnaviridae	Root-knot nematode associated marnavirus 2a	RKNMV2a	BK071954	6873	<i>Meloidogyne incognita</i>	SRR17756052	QKQ15130 hypothetical protein 1 [Forsythia suspensa marnavirus]	59%	79%	0
Marnaviridae	Marnaviridae	Root-knot nematode associated marnavirus 2b	RKNMV2b	BK071955	7559	<i>Meloidogyne incognita</i>	SRR13844590	QKQ15130 hypothetical protein 1 [Forsythia suspensa marnavirus]	63%	74%	0
Marnaviridae	Marnaviridae	Potato cyst nematode associated marnavirus 1	PCNMV1	BK071956	5062	<i>Globodera rostochiensis</i>	SRR1873812	UNY42079 MAG; polyprotein 1, partial [Picornavirales sp.]	99%	77%	0
Marnaviridae	Marnaviridae	Potato cyst nematode associated marnavirus 2	PCNMV2	BK071957	2494	<i>Globodera rostochiensis</i>	SRR1873812	ULF99940 MAG; hypothetical protein [Marnaviridae sp.]	99%	49%	0
Marnaviridae	Marnaviridae	Rice root-knot nematode associated marnavirus 1	RRKNMV1	BK071958	7362	<i>Meloidogyne graminicola</i>	ERR1744941	ULF99981 MAG; hypothetical protein 1 [Marnaviridae sp.]	99%	50%	0
Marnaviridae	Marnaviridae	Cereal cyst nematode associated marnavirus 1	CCNMV1	BK071959	9038	<i>Heterodera avenae</i>	SRR5588564	UNY42137 MAG; polyprotein, partial [Picornavirales sp.]	92%	44%	0
Dicistroviridae	Dicistroviridae	Root-knot nematode associated dicistro-like virus 1	RKNDicV1	BK071960	8484	<i>Meloidogyne incognita</i>	SRR7601332	XCO49623 MAG; putative ORF1 protein, partial [Picornavirales sp.]	41%	45%	0
Dicistroviridae	Dicistroviridae		RKNDicV2	BK071961	9130		SRR17756052		41%	62%	0

Table 1 (continued) | Summary of identified viruses that probably infected or are associated with PPNS

Genome type	Classification	Virus	Abbreviation	Accession	Genome (nt)	Host	Representative SRA	Best hit (BLASTp)	Query coverage	Identity	E-value
Dicistroviridae		Root-knot nematode associated dicistro-like virus 2				<i>Meloidogyne incognita</i>		XCO49623 MAG; putative ORF1 protein, partial [Picornavirales sp.]			
		Root-knot nematode associated dicistro-like virus 3	RKNDicV3	BK071962	6863	<i>Meloidogyne incognita</i>	SRR17756052	ULF99688 MAG; nonstructural protein [Picornavirales sp.]	99%	37%	0
		Root-knot nematode associated dicistro-like virus 4	RKNDicV4	BK071963	8289	<i>Meloidogyne incognita</i>	SRR17756052	UQR78684 nonstructural polyprotein [Striga asiatica dicistro-like virus 1]	100%	82%	0
		Root-knot nematode associated dicistro-like virus 5	RKNDicV5	BK071964	6066	<i>Meloidogyne incognita</i>	SRR17756053	AWK77898 nonstructural polyprotein, partial [Perth bee virus 4]	100%	74%	0
		Root-knot nematode associated dicistro-like virus 6	RKNDicV6	BK071965	8075	<i>Meloidogyne incognita</i>	SRR17756052	AWK77898 nonstructural polyprotein, partial [Perth bee virus 4]	91%	74%	0
		Potato cyst nematode associated dicistro-like virus 1	PCNDicV1	BK071999	6370	Unknown	SRR17221743	XCM67876 MAG; nonstructural polyprotein [Picornavirales sp.]	70%	37%	7E-142
Dicistroviridae		Potato cyst nematode associated dicistro-like virus 2	PCNDicV2	BK072000	3677	Unknown	SRR1873812	XHA87884.1 polyprotein [Qianjiang dicistro-like virus 91]	99%	36%	0
Unclassified picornavirus		Cereal cyst nematode picorna-like virus 1a	CCNPV1a	BK071967	8536	<i>Heterodera avenae</i>	SRR5942326	AVK42881 polyprotein [Potato cyst nematode picorna-like virus]	82%	29%	1.32E-160
Unclassified picornavirus		Cereal cyst nematode picorna-like virus 1b	CCNPV1b	BK071968	8536	<i>Heterodera avenae</i>	SRR5942326	AVK42881 polyprotein [Potato cyst nematode picorna-like virus]	82%	29%	2.99E-161
Unclassified picornavirus		Cereal cyst nematode picorna-like virus 1c	CCNPV1c	BK071969	5396	<i>Heterodera avenae</i>	SRR5942326	WWV87039 MAG; polyprotein, partial [Picornaviridae sp.]	81%	27%	8.96E-136
Unclassified picornavirus		Cereal cyst nematode picorna-like virus 2	CCNPV2	BK071970	4830	<i>Heterodera avenae</i>	SRR5942326	AVK42881 polyprotein [Potato cyst nematode picorna-like virus]	96%	27%	3.4E-123
Unclassified picornavirus		Root lesion nematode virus	RLNV	BK071971	8596	<i>Pratylenchus penetrans</i>	SRR2962755	AYX41642 polyprotein [Root lesion nematode virus]	100%	100%	0
Unclassified picornavirus		Sugar beet cyst nematode virus 1	SBCNV1	BK071966	9515	<i>Heterodera schachtii</i>	SRR16675965	YP_009553470.1 [Sugar beet cyst nematode virus 1]	100%	99%	0
Unclassified picornavirus		Potato cyst nematode picorna-like virus 1	PCNPV1	BK071972	9351	<i>Globodera rostochiensis</i>	ERR1173512	AVK42881 polyprotein [Potato cyst nematode picorna-like virus]	100%	100%	0
Unclassified picornavirus		Potato cyst nematode picorna-like virus 2	PCNPV2	BK071973	8270	<i>Globodera rostochiensis</i>	ERR202481	AVK42881 polyprotein [Potato cyst nematode picorna-like virus]	100%	92%	0
Astroviridae		Potato cyst nematode associated astro-like virus 1	PCNAstV1	BK071974	6837	Unknown	SRR1873815	WZH61648 RNA-dependent RNA polymerase, partial [Sopocug virus]	72%	28%	3.98E-42
Astroviridae		Potato cyst nematode associated astro-like virus 2	PCNAstV2	BK071975	4484	Unknown	SRR1873815	UQB75990 RNA-dependent RNA polymerase [Flumine Astrovirus 8]	70%	22%	4E-13
Astroviridae		Soybean cyst nematode associated astro-like virus 1	SCNAstV1	BK071976	5531	Unknown	SRR9647099	WRQ64769 RNA-dependent RNA polymerase, partial [Patatavirales sp.]	43%	27%	2.6E-28

Table 1 (continued) | Summary of identified viruses that probably infected or are associated with PPNs

Genome type	Classification	Virus	Abbreviation	Accession	Genome (nt)	Host	Representative SRA	Best hit (BLASTp)	Query coverage	Identity	E-value
Unclassified <i>Bormycovirales</i>	Potato rot nematode associated ormycovirus 1	PRNOrmv1-RNA1	PV640412	3322	<i>Ditylenchus destructor</i>	SRR28892574	USW07198 putative RNA-dependent RNA polymerase [Plasmopara viticola lesion-associated ormycovirus 6]	67%	36%	1.14E-134	
Unclassified <i>Bormycovirales</i>	Potato rot nematode associated ormycovirus 1	PRNOrmv1-RNA2	PV640413	1725	<i>Ditylenchus destructor</i>	SRR28892574	No significant hit	61%	37%	2.15E-127	
Unclassified <i>Bormycovirales</i>	Potato rot nematode associated ormycovirus 1	PRNOrmv1-RNA3	PV640414	2061	<i>Ditylenchus destructor</i>	SRR28892574	No significant hit	61%	37%	2.15E-127	
Unclassified <i>Bormycovirales</i>	Soybean cyst nematode associated ormycovirus 3	SCNOrmv3-RNA1	BK071989	3535	Unknown	SRR9647096	USW07198 putative RNA-dependent RNA polymerase [Plasmopara viticola lesion-associated ormycovirus 6]	61%	37%	2.15E-127	
Unclassified <i>Bormycovirales</i>	Soybean cyst nematode associated ormycovirus 3	SCNOrmv3-RNA2a	BK071990	2608	Unknown	SRR9647096	No significant hit	61%	37%	2.15E-127	
Unclassified <i>Bormycovirales</i>	Soybean cyst nematode associated ormycovirus 3	SCNOrmv3-RNA2b	BK071991	2159	Unknown	SRR9647096	No significant hit	61%	37%	2.15E-127	
Unclassified <i>Bormycovirales</i>	Rice root-knot nematode associated ormycovirus 1	RRKNOrmv1-RNA1	BK071983	3299	Unknown	ERR4288691	XDO01625 RNA-dependent RNA polymerase [Phytophthora cinnamomi ormycovirus 7-5]	45%	29%	3.74E-29	
Unclassified <i>Bormycovirales</i>	Rice root-knot nematode associated ormycovirus 1	RRKNOrmv1-RNA2	BK071984	2026	Unknown	ERR4288691	No significant hit	50%	24%	4.61E-26	
Unclassified <i>Bormycovirales</i>	Reniform nematode associated ormycovirus 1	RNOrmV1-RNA1	BK071994	3520	<i>Rotylenchulus reniformis</i>	ERR744557	XDO01625 RNA-dependent RNA polymerase [Phytophthora cinnamomi ormycovirus 7-5]	54%	23%	3.94E-27	
Unclassified <i>Bormycovirales</i>	Reniform nematode associated ormycovirus 1	RNOrmV1-RNA2	BK071995	2336	<i>Rotylenchulus reniformis</i>	ERR744557	No significant hit	54%	23%	3.94E-27	
Unclassified <i>Bormycovirales</i>	Reniform nematode associated ormycovirus 2	RNOrmV2-RNA1	BK071996	3368	<i>Rotylenchulus reniformis</i>	ERR744557	XDO01625 RNA-dependent RNA polymerase [Phytophthora cinnamomi ormycovirus 7-5]	54%	23%	3.94E-27	
Unclassified <i>Bormycovirales</i>	Reniform nematode associated ormycovirus 2	RNOrmV2-RNA2	BK071997	2268	<i>Rotylenchulus reniformis</i>	ERR744557	No significant hit	55%	23%	4.13E-21	
Unclassified <i>Bormycovirales</i>	Soybean cyst nematode associated ormycovirus 1	SCNOrmv1-RNA1	BK071985	3391	<i>Heterodera glycines</i>	SRR13711080	XDO01625 RNA-dependent RNA polymerase [Phytophthora cinnamomi ormycovirus 7-5]	97%	27%	2.13E-11	
Unclassified <i>Bormycovirales</i>	Potato rot nematode associated ormycovirus 3	PRNOrmv3-RNA1	PV640417	715	Unknown	SRR28892574	XDO01621 RNA-dependent RNA polymerase [Phytophthora cinnamomi ormycovirus 5-2]	97%	27%	2.13E-11	
Unclassified <i>Bormycovirales</i>	Potato rot nematode associated ormycovirus 3	PRNOrmv3-RNA2	PV640418	1843	Unknown	SRR28892574	No significant hit	97%	27%	2.13E-11	

Table 1 (continued) | Summary of identified viruses that probably infected or are associated with PPNs

Genome type	Classification	Virus	Abbreviation	Accession	Genome (nt)	Host	Representative SRA	Best hit (BLASTp)	Query coverage	Identity	E-value
	<i>Deltaormyoviridae</i>	Soybean cyst nematode associated ormycovirus 4	SCNOmV4-RNA1	BK071992	2712	Unknown	SRR6269845	WPV08068 MAG: RNA-dependent RNA polymerase [Verticillium dahliae ormycovirus 1]	82 %	30 %	1.07E-81
	<i>Deltaormyoviridae</i>	Soybean cyst nematode associated ormycovirus 4	SCNOmV4-RNA2	BK071993	1424	Unknown	SRR6269845	WPV08069 MAG: hypothetical protein [Verticillium dahliae ormycovirus 1]	90 %	27 %	4.94E-19
	<i>Alphaormyoviridae</i>	Potato rot nematode associated ormycovirus 2	PRNOmV2-RNA1	PV640415	3183	Unknown	SRR28892574	USW07195 putative RNA-dependent RNA polymerase [Erysiphe lesion-associated ormycovirus 1]	36 %	28 %	1.28E-29
	<i>Alphaormyoviridae</i>	Potato rot nematode associated ormycovirus 2	PRNOmV2-RNA2	PV640416	1599	Unknown	SRR28892574	No significant hit			
	<i>Alphaormyoviridae</i>	Soybean cyst nematode associated ormycovirus 2	SCNOmV2-RNA1	BK071987	3102	Unknown	SRR9647098	USW07195 putative RNA-dependent RNA polymerase [Erysiphe lesion-associated ormycovirus 1]	49 %	25 %	3.05E-29
	<i>Alphaormyoviridae</i>	Soybean cyst nematode associated ormycovirus 2	SCNOmV2-RNA2	BK071988	2081	Unknown	SRR9647098	No significant hit			
	<i>Alphaormyoviridae</i>	Potato cyst nematode associated ormycovirus 1	PCNOmV1-RNA1	BK071981	2024	Unknown	ERR202480	USW07200 putative RNA-dependent RNA polymerase [Downy mildew lesion associated ormycovirus 1]	70 %	26 %	1E-20
	<i>Alphaormyoviridae</i>	Potato cyst nematode associated ormycovirus 1	PCNOmV1-RNA2	BK071982	1884	Unknown	ERR202480	No significant hit			
	<i>Alphaormyoviridae</i>	Potato rot nematode associated ormycovirus 4	PRNOmV4-RNA1	BK071979	3131	Unknown	SRR7943144	No significant hit			
	<i>Alphaormyoviridae</i>	Potato rot nematode associated ormycovirus 4	PRNOmV4-RNA2	BK071980	1667	Unknown	SRR7943144	No significant hit			
	<i>Birnaviridae</i>	Potato rot nematode birnavirus 1	PRNBIV1-segmentB	PV640411	2694	<i>Ditylenchus destructor</i>	SRR28892574	QBS55241 polyprotein, partial [Tetranychus urticae-associated entomobirnavirus]	78 %	36 %	1.1E-121
	<i>Birnaviridae</i>	Potato rot nematode birnavirus 1	PRNBIV1-segmentA	PV640410	3340	<i>Ditylenchus destructor</i>	SRR28892574	YP_010086266 RNA-dependent RNA polymerase [Lates calcarifer birnavirus]	85 %	26 %	1.32E-60
	<i>Amalgaviridae</i>	Potato rot nematode associated amalga-like virus 1	PRNAmaV1	PV640419	3312	Unknown	SRR28892574	DAZ85680 TPA_asm: fusion protein [Physcomitrium patens amalgalavirus 1]	88 %	40 %	0
	<i>Amalgaviridae</i>	Soybean cyst nematode associated amalga-like virus 1	SCNAmaV1	BK071998	3911	Unknown	SRR9647096	DAZ85680 TPA_asm: fusion protein [Physcomitrium patens amalgalavirus 1]	92 %	42 %	0

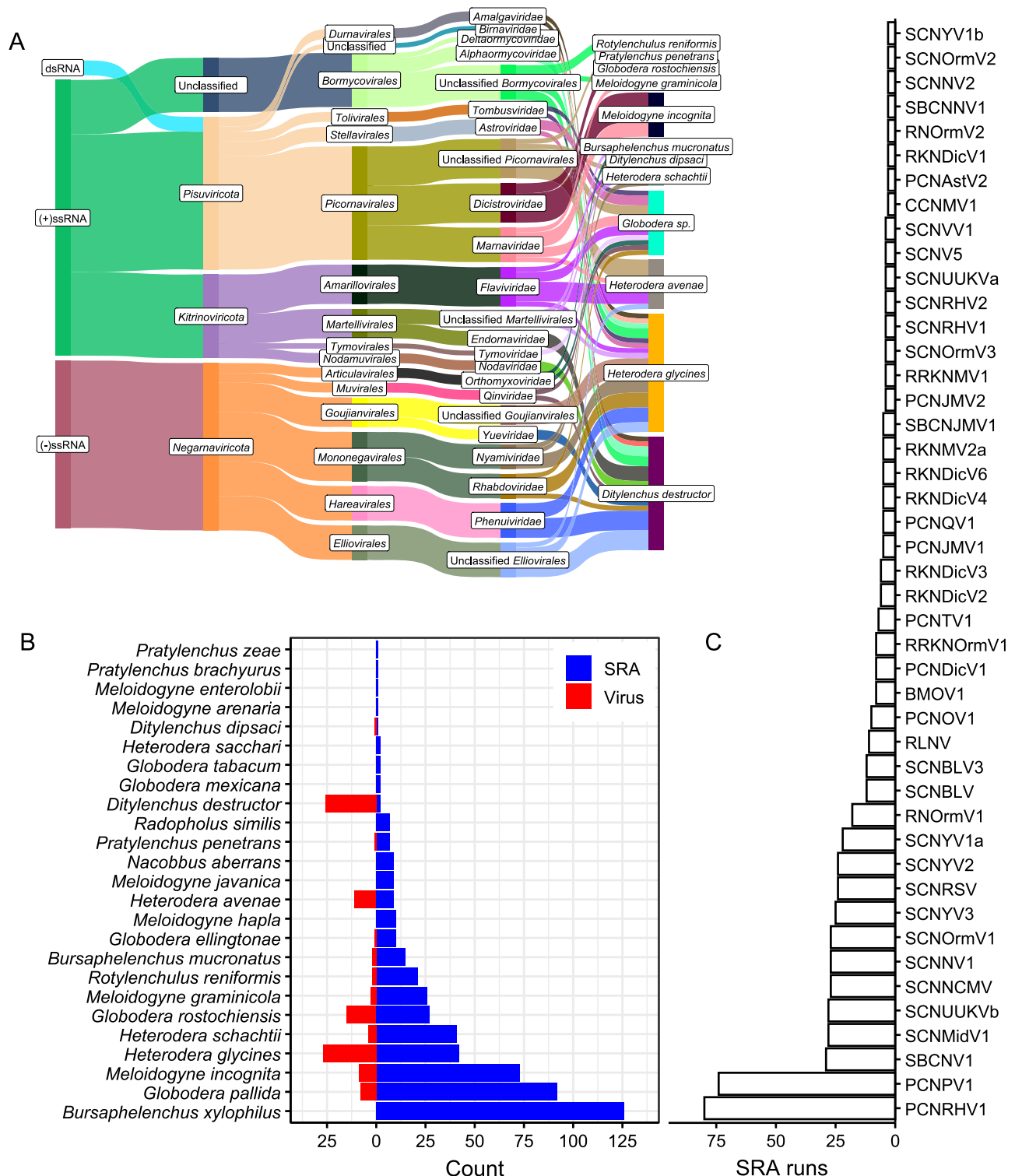


Fig. 1 | Overview of PPN-associated RNA viruses. A Sankey diagram illustrating genome types, viral taxonomy, and hosts of PPN-associated RNA viruses. **B** Back-to-back bar plot comparing the total number of PPN-associated viruses (left) and

corresponding SRA runs across nematode species. **C** Total numbers of SRA run for identified PPN-associated viruses. Only viruses with > 2 SRA runs are shown.

analysis of the RdRP domain in the L protein suggests that SBCNNV1 forms a lineage near SCNMidV1 and is distantly related to another clade containing SCNNV and SCNNV2. This finding suggests that the current *Soccyvirus* genus could be expanded to include these nematode-specific nyami-like viruses (Fig. 2).

Another partial nyami-like virus, SBNNV1, is located at the stem of PPN nyami-like viruses with 100% UFBoot support (Fig. 2). The

percentage of reads assigned to SBNNV1 (0.0009%) is low in the SRR8239758 run of *Ditylenchus dipsaci*, where 91% of reads are classified as *D. dipsaci*, and 5% remain unclassified (Supplementary Note 1). Although SBNNV1, together with *Drosophila Inveresk nyamivir*, appears to cluster closely with the clade of nematode-specific nyami-viruses, it remains unclear whether the host of SBNNV1 is *Drosophila* sp., another insect, or a nematode.

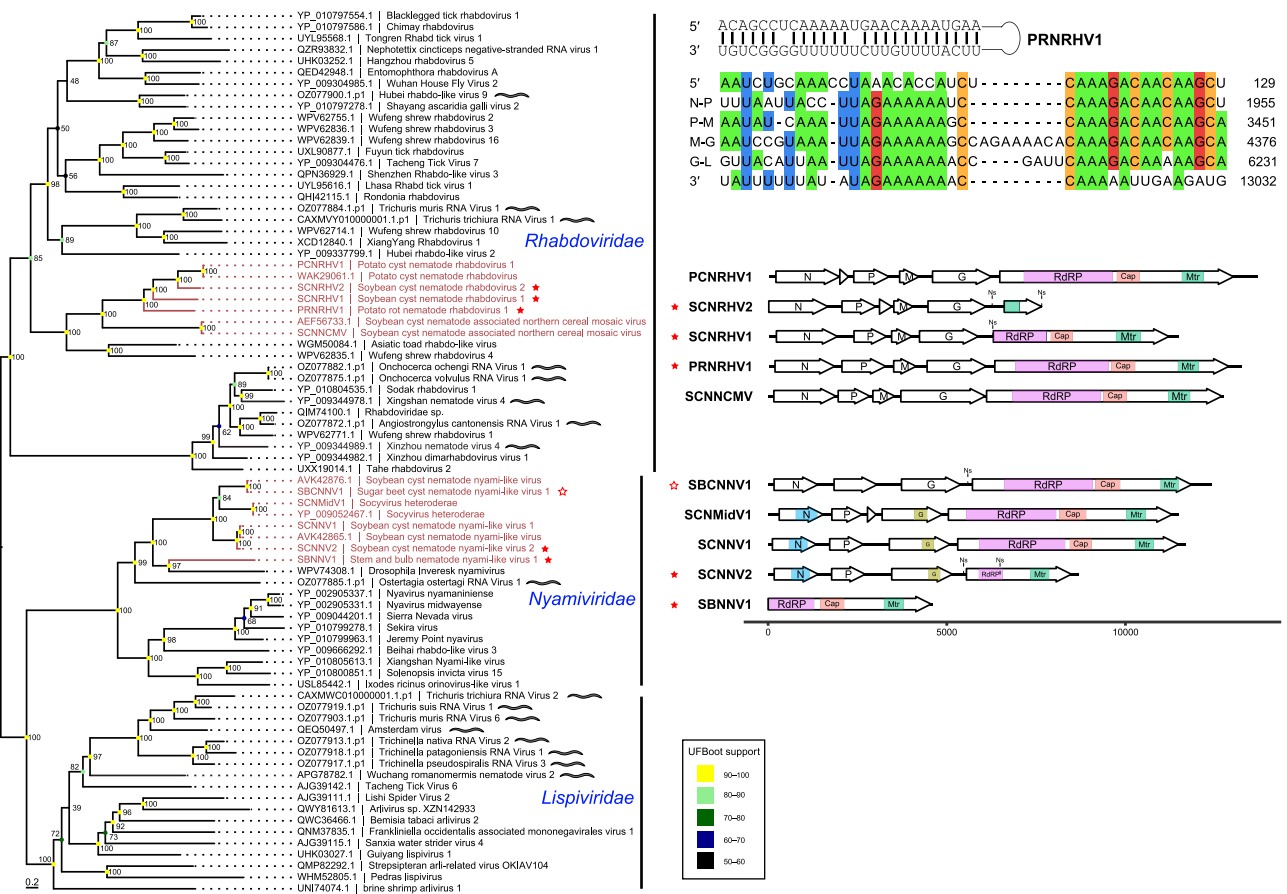


Fig. 2 | NYami-like viruses and rhabdoviruses in PPNs. Phylogenetic tree based on the large (L) proteins of nyami-like viruses and rhabdoviruses is shown at the left panel. The genome organization of PPN-associated nyami-like viruses and rhabdoviruses is shown at the right panel. The complementary of 5' and 3' terminal and the conservation of the intergenomic region of PRNRHV1 are shown. Newly

(ii) Bunyaviricetes. We identified 14 bunya-like viruses, including 11 newly discovered viruses—eight potato rot nematode bunyaviruses (PRNBYV1–8), two soybean cyst nematode bunya-like viruses (SCNBLV2–3), and cereal cyst nematode bunyavirus 1 (CCNBYV1)—as well as three previously reported bunyaviruses: soybean cyst nematode associated Uukuniemi virus (SCNUUKV), soybean cyst nematode associated rice stripe virus (SCNRSV), and soybean cyst nematode bunya-like virus (SCNBLV) (Table 1). Based on phylogenetic analysis of the RdRP domain, seven bunya-like viruses, including previously reported SCNUUKV and SCNRSV, cluster within *Phenuiviridae*. PRNBYV4–6 cluster into a single subclade, and PRNBYV7 forms another subclade with SCNBLV3 and sugar beet cyst nematode virus 2. The remaining bunyaviruses could be grouped into two unknown clades. The first unknown clade includes PRNBYV3, SCNBLV2, and CCNBYV1. Another unknown clade consists of PRNBYV1 (12,325 nt) and PRNBYV2 (12,018 nt), both of which possess an L segment longer than those of SCNBLV (9,478 nt) and most other known bunyaviruses (Fig. 3). Apart from the L_{protein_N} domain (i.e., an endonuclease domain) and the Bunya_RdRp domain, which are typically found in the L protein of most bunyaviruses, we identified a putative cysteine proteinase domain near the C-terminus of the L proteins of PRNBYV1 and PRNBYV2. The conservation of the Asp, Cys, and His catalytic triad, along with the structural similarity observed among PRNBYV1, PRNBYV2, and sentrin-specific protease 8 (SEN8) (Fig. 4A, B), suggests that the cysteine proteinase domains in PRNBYV1 and PRNBYV2 are similar to SEN8, which processes ubiquitin-like proteins. Notably, the SENP-like proteases of PRNBYV1 and PRNBYV2 are phylogenetically

identified viruses in this study are marked with solid red star. The hollow red star represents SBCNNV1, which was previously misnamed as soybean cyst nematode nyami-like virus. Ns, gaps in the assembled viral genome. Animal nematode-associated viruses are followed by a nematode illustration. Q.pfam+F+I+R7 was selected as the best-fit model.

close to sentrin-specific protease 8 of nematodes (Fig. 4C), implying that these viral SENP-like proteases may have been acquired from their hosts. Furthermore, the read-pair coverage among the genomes of PRNBYV1 and PRNBYV2 confirms the continuity of the assembly and rules out the possibility that the SENP-like protease domain resulted from a virus-host chimeric artifact (Fig. 3 and Supplementary Fig. 3). Based on these unique features, PRNBYV1 and PRNBYV2 could be classified into different taxa (e.g., family).

Among PRNBYV1–8, the relative abundances of PRNBYV3–5 were higher than other bunya-like viruses in the library of PRN field nematode populations (SRR28892574) (Supplementary Table 4). Combined with phylogenetic analysis, PRNBYV3–5 and CCNBYV1 are likely hosted by PPNs. As for PRNBYV1–2, they were exclusively and first detected in SRR28892574, where the species composition of the total reads is as follows: *D. destructor* (80%), unclassified reads (12%), *Pristionchus* spp. (8%), *Caenorhabditis* spp. (5%), Viruses (2%), Ascomycota (1%), and Bacteria (1%) (Supplementary Note 1). Given their low abundance (0.005%) and sequence homology to SENP-like protease from *Pristionchus* spp., it remains possible that these viruses may be associated with other contaminants (such as *Pristionchus* spp.) present in the library.

(iii) Articulavirales. (a) Orthomyxoviridae. We identified two new orthomyxo-like viruses, potato cyst nematode orthomyxo-like virus 1 (PCNOV1) and Bursaphelenchus mucronatus orthomyxo-like virus 1 (BMOV1) (Table 1 and Fig. 5). Both PCNOV1 and BMOV1 are composed of five genomic segments, encoding polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2), polymerase acidic protein (PA),

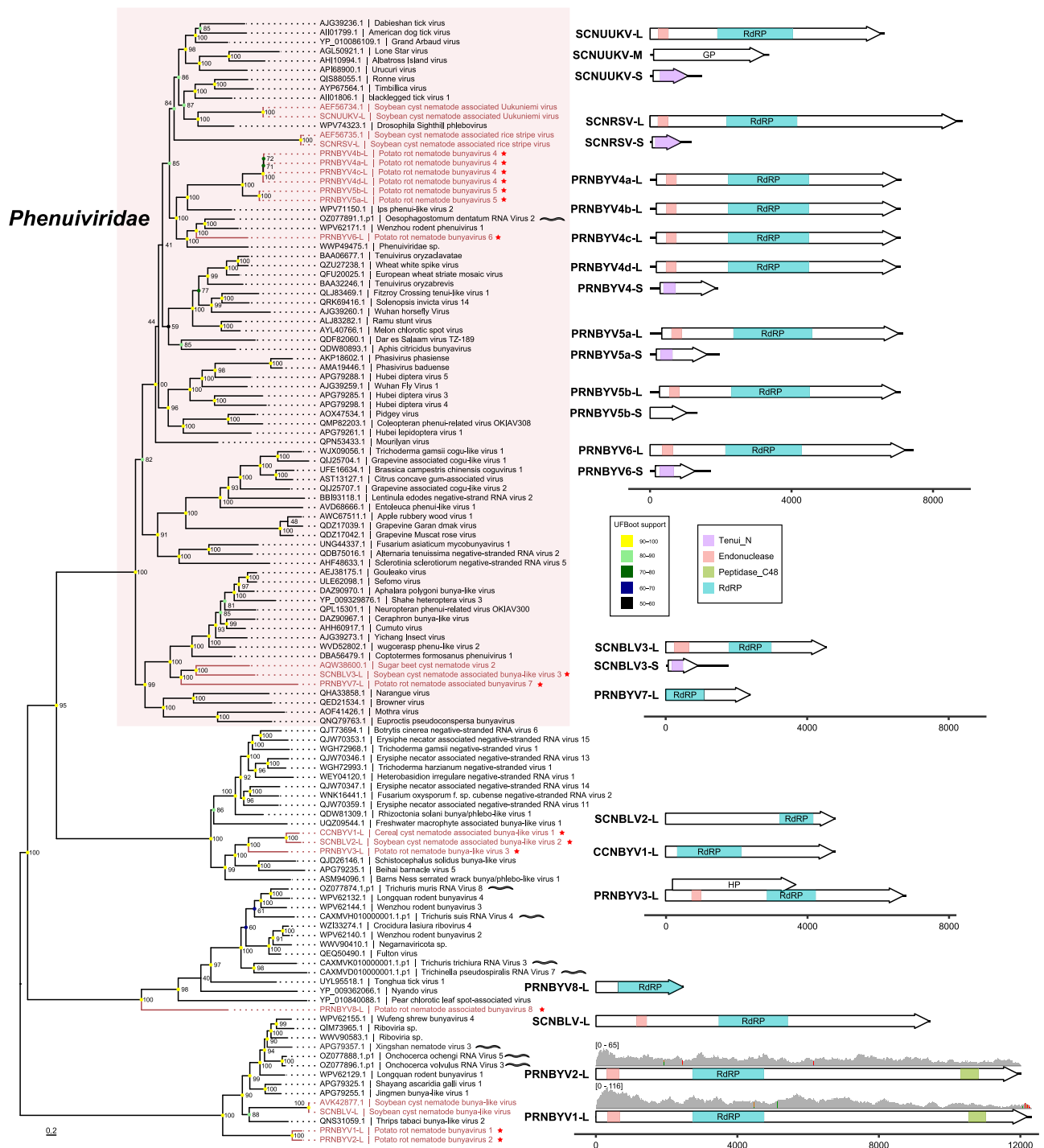


Fig. 3 | Bunya-like viruses in PPNs. Phylogenetic tree based on the RdRp of bunya-like viruses is shown in the left panel. The genome organization of bunya-like viruses is shown in the right panel. The newly identified viruses in this study were labeled with a red star. Ns, gaps in the assembled viral genome. The putative cysteine

protease domain of PRNBYV1 and PRNBYV2 was colored in light-green. Animal nematode-associated viruses are followed by a nematode illustration. Q.pfam +F + I + R9 was selected as the best-fit model.

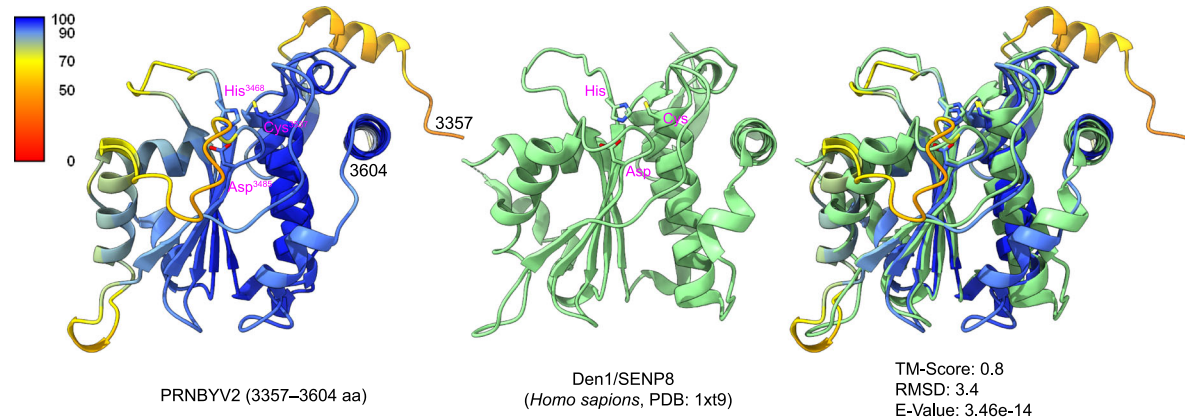
putative glycoprotein (GP), and nucleoprotein (NP) (Fig. 5). PCNOV1 is abundant in seven libraries from two different lineages of *G. pallida* and four different lineages of *G. rostochiensis*; BMOV1 is abundant in eight libraries from three different lineages of pinewood nematodes (*Bursaphelenchus mucronatus*) (Supplementary Fig. 4A). Correlation analysis of segment abundance further indicated that these segments are associated with PCNOV1 and BMOV1 (Supplementary Fig. 4B). The PB1 proteins of PCNOV1 and BMOV1 show the highest similarity (approximately 80% coverage and 40% identity) to the PB1 protein of

Wenling orthomyxo-like virus 2 (AVM87619.1), an orthomyxo-like virus infecting red spikefish, *Triacanthodes anomalus*³⁰. The PA proteins of PCNOV1 and BMOV1 share low similarity with their counterparts from Thailand tick thogotovirus (49% coverage and 27% identity) and Water boatmen thogotovirus 1 (34% coverage and 23% identity), respectively. The PB2, GP, and NP proteins of PCNOV1 and BMOV1 show no detectable sequence similarity to known orthomyxoviral proteins in BLASTP searches. However, HHpred search with the aligned PB2 and NP identified Influenza C virus protein as significant

A

PRNBVY1 Potato rot nematode bunyavirus 1	3454 SVICSYGSQPIFTYFDLSLRD-KQWVAGFIVVAMDYIMIDCLSK-EYLGKVKYLSPAECNYIKYYHETPD---WM-	3523
PRNBVY2 Potato rot nematode bunyavirus 2	3374 FILVSYAEHLTVDFDLSLKK-GSWVEVVIINFMVNYFMDCLPA-NLRDEIAYLTPTDTQILKYRGDI PD--WI-	3443
KAF8376347.1 <i>Pristionchus pacificus</i>	11 SRLLSYGSVVLHTDDVDLLLD-GRWLNRLLTFATEYELMDQLNA-EERERTELVCASTCEMLKFSPEFTS---WL-	80
KAI1721224.1 <i>Ditylenchus destructor</i>	9 SKVLVYDDIVLFGADVNSLIARAGWLTNNVVSFAANYLKNNVVEPKNTDEICIVYASICELIKYCDENEDNSALFK	84
NP_001023477.1 <i>Caenorhabditis elegans</i>	6 DFRLSYESVVL TADDVTILEHTGWFNDKLLTFCAEFLE--QHN-SAAAEIQIFTPPTTEMI RHSTCDEEVDMYFG	78
NP_001159812.1 <i>Homo sapiens</i>	3 PVVLSYMDSLLRQSDVSLLDLP-PSWLNHII GFAFEYFANSQFH-DCSDHVSFISPEVTQFIKCTSNPAEIAMFLE	76
PRNBVY1 Potato rot nematode bunyavirus 1	3524 -EGWDKAEVLIMNVSDCK--PDDQGG-HWSLLVYFAKNNTYHYVDSIYGGNDI-EAKAL---MLMTAP-----	3584
PRNBVY2 Potato rot nematode bunyavirus 2	3444 -QMAMTKKIIILMGI TSSDK--PNDQGG-HWSLLVFNRENNYFYHIDSMTFENGSDTKAC---NQEEAVLLYTVMS	3511
KAF8376347.1 <i>Pristionchus pacificus</i>	81 -SDYRSRSLVFVFNNNRD--PTRPGGSHWSLLIYEKERNRFSYLSLSPDTE-DAEAI---MHAAAP-----	142
KAI1721224.1 <i>Ditylenchus destructor</i>	85 DIGLSPEKWCFFILNDNADPNVAYGGS-HWCLLLFDPLKQSFLLFDSMPGSDYGSVQSDIISFASKLKK	152
NP_001023477.1 <i>Caenorhabditis elegans</i>	79 CLDVNSKDLIAFIVNDNQDVTNRVNGGS-HWSLLIFDRKIDKFRHFDSSARGHNLK-I AENL---MQKSRK-----	142
NP_001159812.1 <i>Homo sapiens</i>	77 PLDLPNKRVLFLAINDNSN--QAAGGT-HWSLLVYLQDKNSFFHYDSHSRSNSV-HAKQV---AEKLEA-----	138
PRNBVY1 Potato rot nematode bunyavirus 1	3585 -YLCKDGNTPSFLTPICPROSGTDCGMYLVEHVRYILQYCLEDEIEGGFEKLAHLKLDLSVAIMEARLNWRLLIVRL	3659
PRNBVY2 Potato rot nematode bunyavirus 2	3512 ALSLNQGEYPTYLTPICPQQTNGNDCGMYLIEHARYVMQNCLEDMMHNGIMKLKYYIQI GEPEIMESRYCMRKIIAGM	3587
KAF8376347.1 <i>Pristionchus pacificus</i>	143 -HLCPPGT KPLRLHTLTCPKQENTNDCGVMYIEFVRFMLOHGRKSAAKALDMLACTRMDGAYMKDCRNWRTRISDL	217
KAI1721224.1 <i>Ditylenchus destructor</i>	153 -FLCCKAE-LLVEAVKCPKMSISGDCGMFVIEYMRVNLNALTNIIRLDSHKIDLSHINDKFI LSRQQTWWFLIKDL	226
NP_001023477.1 <i>Caenorhabditis elegans</i>	143 -LVRQRQSRNLEPELCLQKNASDCGLHVAQYLAVALAE-----QKSD--AIKLEKIDKTRGFWRDLIVNF	207
NP_001159812.1 <i>Homo sapiens</i>	139 -FLGRKGDKLAFVEEKAPQNSYDCGMVYICNTEALCQ---NFFRQQTESLLQLLTPAYITKKRGEWKDLITTL	209

B



C

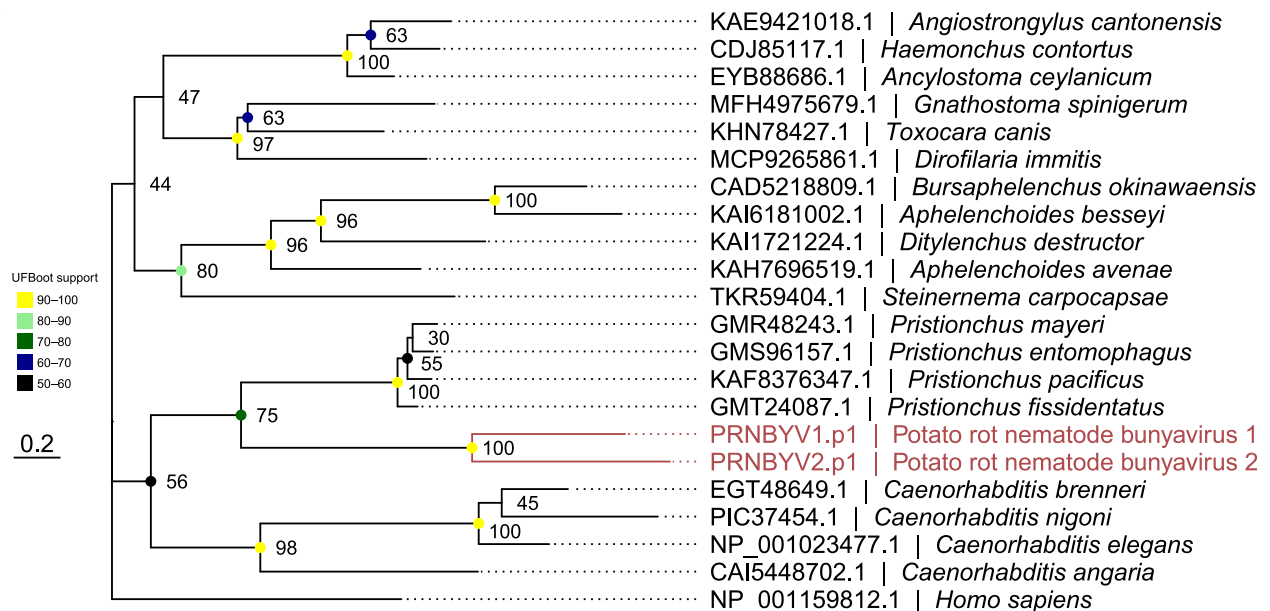


Fig. 4 | SENP-like proteases of PRNBVY1 and PRNBVY2. A Alignment of SENP-like proteases from PRNBVY1 and PRNBVY2 with the nematode and human homologs of SENP8. The putative catalytic triad (His-Asp-Cys) was marked with red circles. **B** Structural comparison of the SENP-like protease from PRNBVY2 and the human Den1/SEN8. The structure of SENP-like protease from PRNBVY2 was predicted with ColabFold and colored according to the pLDDT value. The human

Den1/SEN8, shown in light-green, was identified as the most similar structure of SENP-like proteases from PRNBVY2 in the PDB database by Foldseek search. The TM-score, RMSD, and E-value were calculated with the Foldseek webserver. All the protein structure graphics were visualized with ChimeraX. **C** Phylogenetic tree of the SENP-like proteases from PRNBVY1 and PRNBVY2. The tree was constructed using IQ-TREE2 with “LG + I + G4” as the best-fit model and visualized using ggtree.

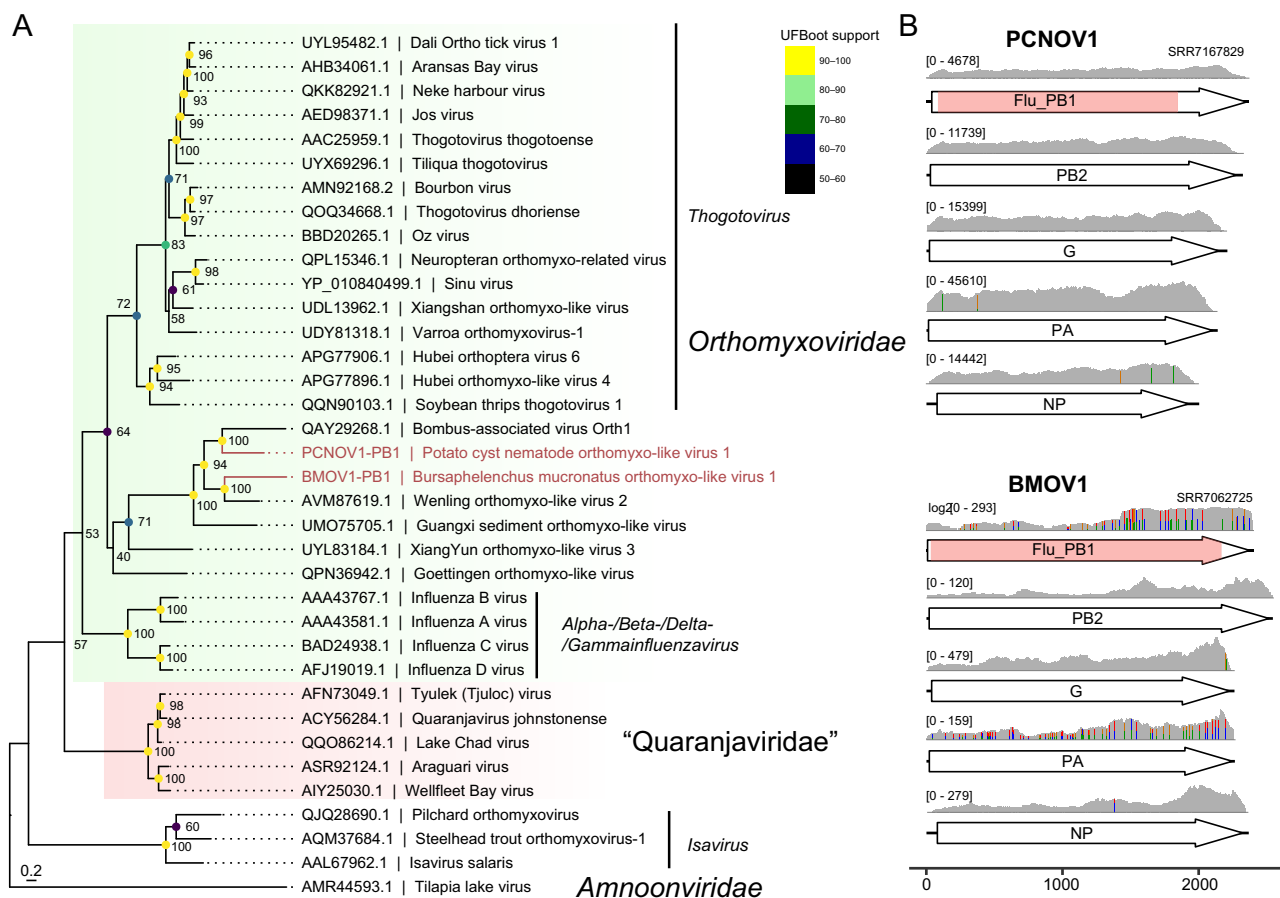


Fig. 5 | Orthomyxo-like viruses in PPNs. A Phylogenetic tree based on the main replicase PB1 of *Articulavirales*. Q.pfam+F+I+R5 was selected as the best-fit model. **B** Genome organization of BMOV1 and PCNOV1. Read coverage profiles,

generated using Bowtie 2 (version 2.5.4), were visualized alongside the viral genome. Coverage value ranges are indicated in square brackets. Note that coverage across the 5' region of BMOV1-PB1 is low and is therefore displayed on a logarithmic scale.

match, with probabilities exceeding 95% and E-values below 3.7×10^{-5} (Supplementary Fig. 5). In contrast, the aligned GP sequences of PCNOV1 and BMOV1 show similarity to those of mononegavirals (e.g., Borna disease virus), with probabilities above 99% and E-value lower than 1.5×10^{-23} , than to the hemagglutinins of known articularvirals (Supplementary Fig. 5). Phylogenetic analysis of PB1 shows that PCNOV1 and BMOV1 form a well-supported clade (94% UFBoot support) with some insect/fish-associated orthomyxo-like viruses, which represents a sister clade of thogotovirus in *Orthomyxoviridae* (Fig. 5).

Among the SRA runs where PCNOV1 was detected, PCNOV1 is most abundant in three SRA runs, SRR16693885, SRR7167829, and ERR1173512, where the contaminant reads are below 1%. For BMOV1, the contaminant reads in SRR7062725 and SRR7062722 are below 2% (Supplementary Note 1). These analyses suggest *Globodera* spp. and *B. mucronatus* as the probable hosts for PCNOV1 and BMOV1, respectively.

(iv) Goujianvirales. (a) Yueviridae and (b) Qinviridae. We identified six yue-like viruses: potato rot nematode yue-like virus 1 (PRNYV1), potato rot nematode yue-like virus 2 (PRNYV2), soybean cyst nematode yue-like viruses 1a (SCNYV1a), soybean cyst nematode yue-like viruses 1b (SCNYV1b), soybean cyst nematode yue-like viruses 2 (SCNYV2), and soybean cyst nematode yue-like viruses 3 (SCNYV3) (Table 1 and Fig. 6A). Except for PRNYV2, which contains three segments, all five other yue-like viruses comprise two genome segments. The large segment (RNA1) of all PPN-associated yue-like viruses encodes a protein with an RdRP domain. The small segment (RNA2) encodes a hypothetical protein. The RNA2 and RNA3 of PRNYV2 share partial nucleotide similarity, especially at the 5' and 3' terminal regions (Fig. 6A), and their

encoded proteins share 43% aa identity. Furthermore, while the RNA2 of PRNYV1 shows conservation with known yueviruses in *Yueviridae*, the RNA2 of PRNYV2, SCNYV1a, SCNYV1b, SCNYV2, and SCNYV3 are longer and exhibit no significant sequence similarity or homology to the former. In the RNA1-based phylogenetic tree, PRNYV1 clusters within the established *Yueviridae* family, while the remaining five yue-like viruses—PRNYV2, SCNYV1a, SCNYV1b, SCNYV2, and SCNYV3—form a distinct monophyletic clade. Within this clade, PRNYV2 groups with Thrips tabaci-associated yue-like virus 1 (Ttaye1)³¹, whereas the four SCNYVs (SCNYV1a, SCNYV1b, SCNYV2, and SCNYV3) comprise a separate, well-supported subclade (Fig. 6A).

Unexpectedly, the coding regions of RNA1 of the four SCNYVs, as well as RNA2 of SCNYV2 and SCNYV3, are interrupted by 1–10 introns containing canonical RNA splice site motifs (GU/AG) (Fig. 6B–C and Supplementary Fig. 6). Furthermore, all SRA runs in which the four SCNYVs were sufficiently covered exhibited clear evidence supporting the presence of these introns (Supplementary Fig. 7). However, no introns were detected in the genomes of Ttaye1 and PRNYV2, despite their close phylogenetic relationship with these intron-bearing yue-like viruses (Supplementary Fig. 6A and Supplementary Fig. 8). Interestingly, although the RNA1 of SCNYV1a and SCNYV1b share 97% aa identity (100% coverage) and 88% nucleotide identity (98% coverage), their intron distributions differ, with only two introns in RNA1 of SCNYV1b but ten in SCNYV1a. To investigate whether the four SCNYVs originated from DNA sequences, such as endogenous viral elements or retro-like viral elements, we performed a BLASTN search using default parameters against the Nucleotide collection (nr/nt), High Throughput Genomic Sequences (HTGS), and Whole Genome Shotgun contigs (WGS) databases, with the latter two restricted to

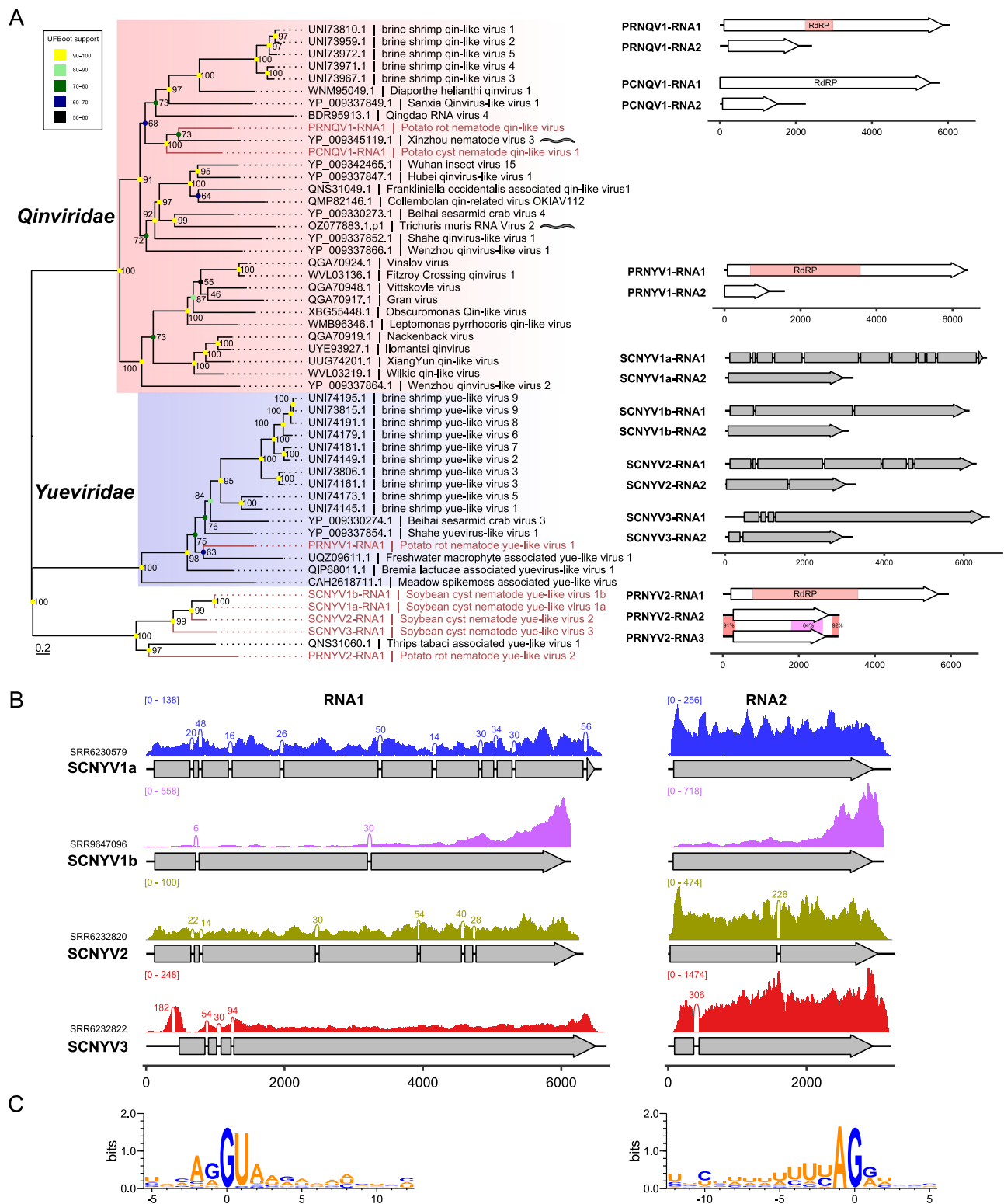


Fig. 6 | Qin- and yue-like viruses. **A** Phylogenetic tree based on the RdRP of qin-like and yue-like viruses is shown at the left panel. The genome organization of qin-like and yue-like viruses is shown at the right panel. In the midpoint-rooted phylogenetic tree, all nematode-associated viruses identified in this study are highlighted in red, and previously reported nematode-associated viruses are followed by a nematode illustration. For the four yue-like viruses with introns, the

exons are represented by gray boxes. Q.pfam+ F + R5 was selected as the best-fit model. **B** Sashimi plot, incorporating read coverage profiles, displays reads spanning the predicted intron-bearing yue-like viruses. The number of reads supporting each splice junction is indicated on the corresponding arc. **C** The conserved nucleotide motif of predicted splice sites identified in four intron-bearing yue-like viruses.

sequences of *Heterodera* spp. No significant hits were obtained. This result suggests that SCNYV1a, SCNYV1b, SCNYV2, and SCNYV3 are likely a unique group of RNA viruses harboring multiple introns within their genomes, a feature rarely observed in RNA viruses.

We identified two bi-segmented qin-like viruses: potato rot nematode qin-like virus 1 (PRNQV1) in the PRN virome, and potato cyst nematode qin-like virus 1 (PCNQV1) in four transcriptome libraries of *G. rostochiensis* derived from three different biosamples in NCBI. Based on phylogenetic analysis of RdRP encoded by RNA1, the two PPN qin-like viruses cluster with Xinzhou nematode virus 3 discovered from snake-associated nematodes, and they form a nematode-infecting virus clade in *Qinviridae* (Fig. 6).

Based on the species composition of the SRA runs associated with the highly abundant viruses—including PCNQV1 (ERR1173512), SCNYV1a (SRR6232813 and SRR6232824), SCNYV1b (SRR9647096), SCNYV2 (SRR6230580), and SCNYV3 (SRR6230583)—the hosts of all identified yue-like and qin-like viruses are likely the respective plant-parasitic nematodes (PPNs).

Positive-sense single-stranded RNA viruses

(i) *Martellivirales*. (a) *Endornaviridae*. We identified three endornaviruses from RNA-seq data of field-collected PRN (Table 1). They share 90–92% nucleotide identity and 95–96% protein identity with each other, and were temporarily named potato rot nematode endornavirus 1a (PRNEV1a), PRNEV1b, and PRNEV1c, representing different variants of a new endornavirus (PRNEV1). PRNEV1 consists of a large ORF encoding a putative polyprotein with the conserved domains of helicase and RdRP (Supplementary Fig. 9). The RdRP phylogenetic tree placed PRNEV1 in the *Alphaendornavirus* genus of *Endornaviridae* (Supplementary Fig. 9). Whether the host of PRNEV1 is indeed PRN remains uncertain, as approximately 1% of the reads in SRR28892574 are attributed to ascomycete fungi which are generally the hosts of endornaviruses (Supplementary Note 1).

(b) *Mycoalphaviridae*. We identified two mycoalphaviruses: soybean cyst nematode-associated alpha-like virus 1 (SCNAlphaV1) and cereal cyst nematode-associated alpha-like virus 1 (CCNAlphaV1), belonging to *Mycoalphaviridae*, which comprises of fungal alpha-like viruses (Supplementary Table 2). Phylogenetic analysis shows that SCNAlphaV1 clusters with an alpha-like virus isolated from the fungus *Leptosphaeria biglobosa* (incorrectly annotated as *Leptosphaeria biglobosa* flavi-like virus 1 in GenBank) (Supplementary Fig. 9). CCNAlphaV1 belongs to the same virus species as *Solanum melongena* bastro-like virus and *Plasmopara viticola* lesion-associated alpha-like virus 1. In the SRA run (SRR5588565) where CCNAlphaV1 was identified, nearly 0.8% of the total reads were classified as Oomycota, and 7% were unclassified. This suggests that CCNAlphaV1 is hosted by contaminated oomycetes or other organisms. Notably, SCNAlphaV1 was identified at relative abundances of 0.01% and 0.04% in the total reads from two SRA runs, SRR9647096 (egg stage) and SRR9647098 (second-stage larva), respectively. Fungal contamination is minimal, comprising only 0.07% of the total reads from these two SRA runs. Furthermore, two previously reported soil-associated nematode viruses, Maryland hepe-like virus 10 and Maryland martelli-like virus 11²⁷, are positioned in *Mycoalphaviridae* (Supplementary Fig. 9). These results imply the close relationships between the nematodes and some fungal mycoalphaviruses.

(c) Unclassified *Martellivirales*. We identified three new viruses belonging to unclassified taxa within *Martellivirales*, comprising sugar beet cyst nematode-associated virga-like virus 1 (SBCNVV1), soybean cyst nematode-associated virga-like virus 1 (SCNVV1), and *Bursaphelenchus mucronatus*-associated virga-like virus (BMVV1) (Supplementary Fig. 9 and Table 1). The genome of BMVV1 is 9,651 nt in length and is predicted to encode a single large ORF exceeding 3000 aa, featuring an Mtr-S1H-RdRP domain arrangement but lacking any ORF encoding capsid proteins. Regarding SBCNVV1 and SCNVV1, although the assembled genome of SBCNVV1 contains two gap regions, the RdRP domains of these two

viruses share approximately 87% aa identity. Additionally, a methyltransferase domain (PfsJ-Mtr), which is usually found in the order *Flasuviricetes*, was identified upstream of the S1H domain in SCNVV1. The second ORF of SBCNVV1 and SCNVV1 encodes a putative capsid protein, sharing low similarity (82% coverage and 24% identity) to a hypothetical protein of animal-infecting nematodes (*Trichinella* spp.). SBCNVV1 and SCNVV1 share a close evolutionary relationship but cluster unreliably with other known virga-like viruses. In contrast, BMVV1 forms a well-supported clade (UFboot support value > 98%) with the plant-associated tobamo-like virus 1 (PaToLV1) and two animal nematode viruses: *Brugia malayi* RNA virus 1 (BmRV1) and *Haemonchus contortus* RNA virus 2. However, BMVV1 shares low sequence similarity (27% coverage and 37% aa identity) with PaToLV1 (Supplementary Fig. 9 and Table 1). The genome of BMVV1 (9.65 kb), which is longer than those of PaToLV1 (6.55 kb) and BmRV1 (7.83 kb), differs markedly from these viruses by encoding only a large polyprotein^{28,32}. These differences suggest that BMVV1 is distinct from PaToLV1 at least at the genus level.

In the SRA runs where SCNVV1 was detected, the reads of SCNVV1 constitute 0.1% and 0.04% of the total reads in SRR9647096 and SRR9647098, respectively, with contaminant reads representing less than 1% of the total reads in both runs (Supplementary Note 1). Based on these analyses, we hypothesized that the host of SCNVV1 is likely SCN. Given the close phylogenetic relationships between SBCNVV1 and SCNVV1, SBCNVV1 may be hosted by SBCN. Among the SRA runs where BMVV1 was detected, the reads of BMVV1 are most abundant in SRR7062723 and account for 0.002% of the total reads. In this library, 80% of the reads are classified as *B. mucronatus*, 13% as Bacteria, and 4% remain unclassified. Additionally, 0.8% of the reads are associated with plants belonging to the Magnoliopsida, including species of *Trifolium*, *Medicago*, and *Citrus*. Furthermore, BMVV1 was only detected in SRA runs derived from egg and L2 stages, but not in L3 & L4 or adult stages (Supplementary Note 1). Based on these results, we cannot exclude the possibility that BMVV1 may be hosted by plants, similar to cycas necrotic stunt virus, which was also identified in the SRR7062723 run.

(ii) *Tymovirales*. We identified eleven tymovirals from PPN-associated SRA datasets, including eight new viruses: potato cyst nematode-associated tymovirus 1 (PCNTV1), soybean cyst nematode-associated gammaflexivirus (SCNGFV1), three soybean cyst nematode-associated deltaflexiviruses (SCNDFV1–3), and three pinewood nematode-associated deltaflexiviruses (PWNDFV1–3) (Table 1 and Supplementary Table 2). The remaining three included two characterized plant viruses—potato virus S (PotVS) and Citrus yellow vein clearing virus (CYVCV)—along with *Agrostis stolonifera* deltaflexivirus 1 (AsDFV1), which has been reported in plants and fungi, respectively. PotVS was found in two SRA runs (ERR202422 and SRR3162514), where no plant-derived reads were detected, implying that potato cyst nematodes may serve as vectors for PotVS transmission. Phylogenetic analysis reveals that PCNTV1 clusters with plant viruses within *Tymoviridae*, and that SCNGFV1, SCNDFV1–3 and PWNDFV1–3 cluster with mycoviruses in *Gammaflexiviridae* and *Deltaflexiviridae*, respectively (Supplementary Fig. 10). In the ERR202430 run, where PCNTV1 is more abundant compared to other SRA runs, no plant-derived reads were identified, except perhaps in the 3% unclassified reads (Supplementary Note 1). This suggests that PCNTV1 may infect or be transmitted by PCNs. As for SCNGFV1, SCNDFV1–3 and PWNDFV1–3, whether SCNs serve as hosts for these remains undetermined.

(iii) *Amarillovirales*. (a) *Flaviviridae*. Eight flaviviruses were discovered in PPN-associated public RNA-seq datasets. Among these, one was soybean cyst nematode virus 5 (a previously reported large genome flavivirus), while the remaining seven were new Jingmen-like viruses, including sugar beet cyst nematode Jingmen virus 1 (SBCNJMV1), two potato cyst nematode Jingmen viruses (PCNJMV1–2), and four cereal cyst nematode Jingmen viruses (CCNJMV1–4) (Table 1 and Fig. 7). Jingmen viruses are characterized by their segmented genomes, which were thought to have evolved from an ancestral, unsegmented

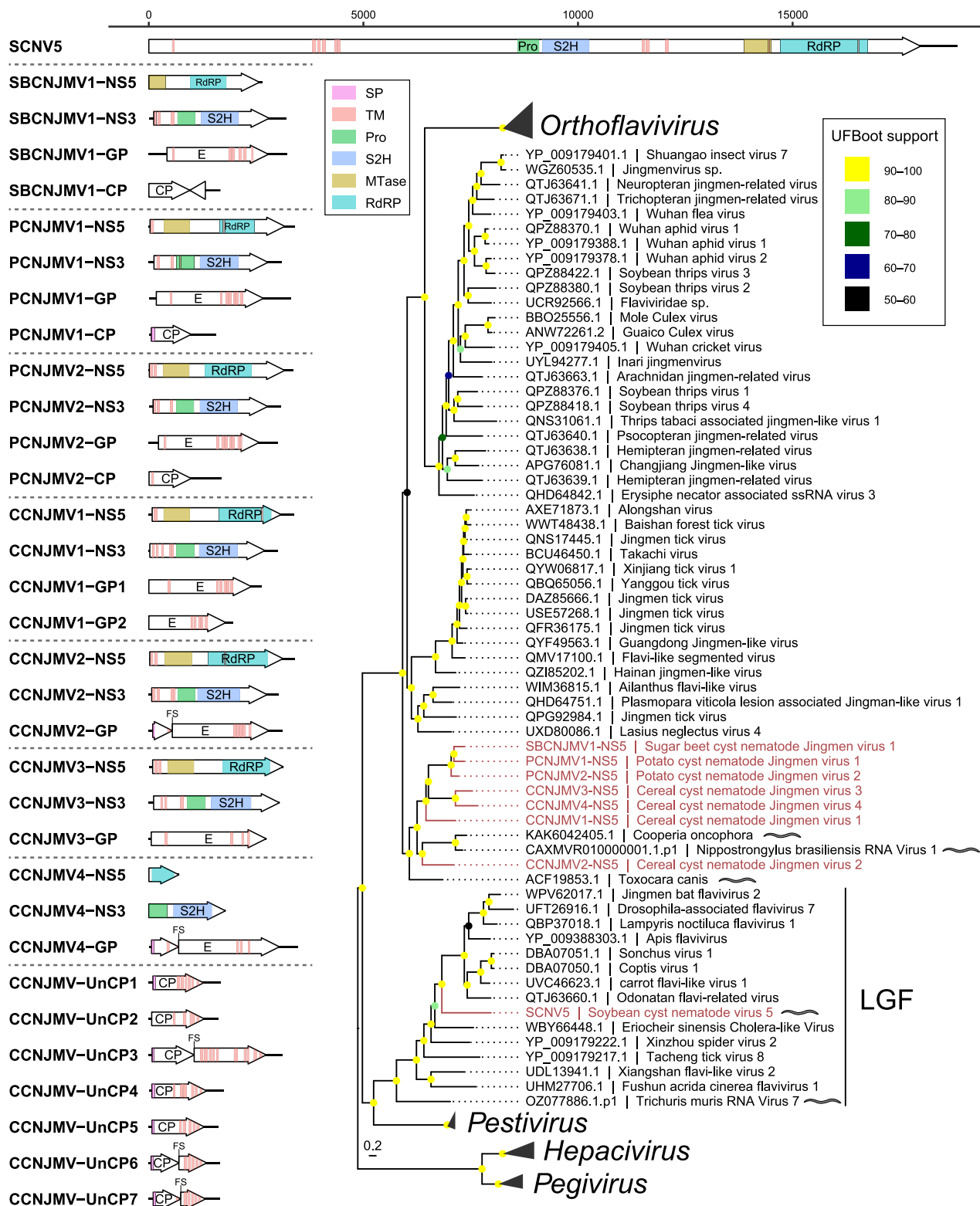


Fig. 7 | Nematode-associated viruses in Flaviviridae. The genome diagram of PPN-associated flavivirus and phylogenetic tree of the members of Flaviviridae based on the RdRP domain in NS5 protein. In the midpoint-rooted phylogenetic tree, all nematode-associated viruses identified in this study are highlighted in red, and previously reported nematode-associated viruses are followed by a nematode

illustration. Q.pfam+F+R7 was selected as the best-fit model for phylogenetic analysis. SP, signal peptide; TM, transmembrane domain; Pro, protease; S2H, superfamily 2 helicase; E, putative envelope protein (glycoprotein); MTase, methyltransferase; UnCP, unassigned capsid segment; FS, putative ribosomal frameshift; LGF, large-genome flavivirus.

flavivirus³³. For the seven newly identified Jingmen viruses, two RNA segments are well-conserved among all known Jingmen viruses: one encoding the non-structural protein 5 (NS5) with a methyltransferase and RdRP domain, and another encoding NS3 proteins with a serine protease and DEAD-like helicase domain. The third segment encodes a putative glycoprotein that likely functions as a viral envelope protein, characterized by a cysteine-rich domain and multiple transmembrane domains (Supplementary Fig. 11). The fourth segment encodes a protein of approximately 400 aa, which includes a conserved motif and features either a signal peptide or a transmembrane domain at its N-terminus, suggesting that the protein may function as a capsid protein (Fig. 7 and Supplementary Fig. 12). However, the presence of multiple Jingmen virus infections (CCNJMV1–4) within SRR5942326 run complicates definitive assignment of genome segments to the corresponding viruses. Based on transcript abundance and phylogenetic analysis, CCNJMV2 is the most abundant, followed by CCNJMV3, which clusters closely with CCNJMV4 (Supplementary Fig. 13). Furthermore, seven distinct putative capsid segments were identified in SRR5942326, adding further complexity to the assignment of these segments to a specific CCNJMV. Ultimately, only three RNA segments could be confidently assigned to CCNJMV1–4.

The NS5 and NS3 proteins of PPN-infecting Jingmen viruses exhibit 26–40% aa identity with previously identified Jingmen viruses. In contrast, glycoproteins and putative capsid proteins of these PPN-infecting Jingmen viruses show no detectable similarity to other known Jingmen viruses (Table 1). A phylogenetic tree of the RdRP domain in the NS5 segment reveals that the seven PPN-infecting Jingmen viruses form a single clade with three putative Jingmen viruses associated with animal-parasitic nematodes: *Cooperia oncophora*, *Nippostrongylus brasiliensis*, and *Toxocara canis*. This phylogenetic evidence supports the classification of this nematode-associated Jingmen virus clade as a novel third lineage closely related to the canonical tick-borne Jingmen tick viruses.

Based on the taxonomic classification of reads from the SRA runs of PCNJMV1 (ERR1173511 and ERR1173512), PCNJMV2 (ERR202480), and SBCNJMV1 (SRR16675965 and SRR16675966), the low proportion of unclassified reads and the absence of contaminants corresponding to known hosts of Jingmen viruses suggest that PPNs likely serve as the hosts of these PPN-specific Jingmen viruses (Supplementary Note 1). Although CCNJMV1–4 were only identified in the SRR5942326 run, which contains a large proportion (38%) of unclassified reads (Supplementary Note 1), phylogenetic analysis supports that these four viruses may be hosted by cereal cyst nematodes or other nematodes.

(iv) Tolivirales. (a) Tombusviridae. We identified two new tombus-like viruses, potato cyst nematode associated tombus-like virus 1 (PCNTomV1) and soybean cyst nematode associated tombus-like virus 1 (SCNTomV1). Phylogenetic analysis shows that PCNTomV1 and SCNTomV1 are placed in two different unclassified clades relative to *Tombusviridae* (Supplementary Fig. 14). SCNTomV1 accounts for approximately 0.03% of the reads in the SRR6269844 run, which include contaminants such as Bacteria (4%), *Phytophthora* spp. (0.9%), Streptophyta (0.4%), and Sporadotrichida (0.3%) (Supplementary Note 1). PCNTomV1 accounts for 0.001% of the reads in the SRR1873823 run, which contain contaminants including Mammalia (8%), unclassified reads (7%) and *Acanthamoeba* spp. (0.4%) (Supplementary Note 1). Based on the taxonomic classification of reads and phylogenetic analysis, whether SCNTomV1 and PCNTomV1 are hosted by PPNs remains unknown.

(v) Nodamuvirales. (a) Nodaviridae. We identified two nodaviruses with bi-segmented RNA genomes in nematode populations isolated from rotting sweet potato. One shares 90–92% identity and over 98% query coverage with RNA1 and RNA2 of Santeuil virus found in *C. briggsae*, and was considered a new variant of the Santeuil virus, hence named Santeuil virus isolate hongshu (Table 1 and Supplementary Fig. 15A). The second nodavirus, named Lulong nodavirus, has a bi-segmented RNA genome of

3452 and 2476 nt in length, respectively. RNA1 was predicted to encode two ORFs: the large one containing a methyltransferase domain and an RdRP domain which shares 32.15% identity with mantis virus (Sanya nodavirus 1, MZ209970.1); and the small one with no predicted domain, located near the 3' terminal of RNA1, and may be translated from a subgenomic RNA as reported in *Alpha-* and *Betanodavirus*. RNA2 of Lulong nodavirus contains two ORFs: ORF1 encoding capsid proteins sharing homology to Capsid-VNN (viral nervous necrosis) of betanodaviruses; and ORF2 predicted to be translated via a ribosomal frame-shifting strategy to express a fusion protein that merges with the capsid of ORF1 (Table 1 and Supplementary Fig. 15A–B). This is similar to the strategy used by the capsid-delta protein encoded by Santeuil virus, but the hypothetical protein of ORF2 does not show homology to the delta protein. In the phylogenetic tree of the RdRP domain of nodaviruses, Lulong nodavirus is grouped with unclassified nodaviruses with low UFBoot support (53%) (Supplementary Fig. 15C). However, the Capsid-VNN domain of Lulong nodavirus seems to be a sister clade of nematode-associated viruses, but with low bootstrap support (Supplementary Fig. 15D). Considering the uncertainty in phylogeny and the low abundance of Lulong nodavirus, we speculate that nematodes may not be their natural hosts, but rather other contaminating organisms in the SRR28892574 run, as mentioned previously. Additionally, due to the presence of approximately 2% reads assigned to *C. briggsae*, it is difficult to ascertain whether Santeuil virus isolate hongshu is from *C. briggsae* or *D. destructor*. Unexpectedly, we found RNA2 of Santeuil virus may be present in a small amount in the circular form, which was confirmed by RT-PCR and read mapping (Supplementary Fig. 16). However, whether the circularity is derived from template-switching or mis-priming during RT-PCR remains to be further determined.

(vi) Picornavirales. (a) Marnaviridae. Marnavirids typically have mono- or dicistronic genome organizations, with non-structural proteins preceding the structural proteins³⁴. Our study identified seven new viruses within the *Marnaviridae* family: rice root-knot nematode associated marnavirus 1 (RRKNMV1), cereal cyst nematode associated marnavirus 1 (CCNMV1), root-knot nematode marnavirus 1 (RKNMV1), two variants of root-knot nematode associated marnavirus 2 (RKNMV2), and potato cyst nematode marnavirus 1–2 (PCNMV1–2) (Table 1). Only a partial genome of PCNMV2, containing regions encoding the capsid protein, was assembled. RKNMV1 and CCNMV1 are monocistronic, while the other four nematode associated marnaviruses are dicistronic, separated by intergenic regions, or they utilize stop-codon readthrough or program frameshift strategies. Phylogenetically, all six nematode-associated marnaviruses are scattered on a branch containing *Locarnavirus* within the *Marnaviridae* family (Fig. 8A and Supplementary Fig. 17).

Most well-known hosts of marnaviruses are organisms in Stramenopiles. Because most marnavirids were discovered in diverse water or water-associated viromic studies, the hosts of these marnavirids remain uncertain. Among the SRA runs in which the six newly identified nematode-associated marnaviruses are abundant, most contain many unclassified reads. The hosts of these marnaviruses discovered in PPN-associated SRA runs remain uncertain (see Supplementary Note 1).

(b) Dicistroviridae. We identified nine dicistroviruses or dicistro-like viruses, including eight new ones: root-knot nematode-associated dicistro-like virus 1–6 (RKNDicV1–6), potato cyst nematode-associated dicistro-like virus 1–2 (PCNDicV1–2), and rice root-knot nematode-associated dicistrovirus 1 (RRKNDicV1) (Table 1 and Supplementary Table 2). RRKNDicV1 exhibits 100% coverage and over 96% identity to Dicistroviridae sp. and an aphid virus—*Rhopalosiphum padi* virus (Supplementary Table 2). Phylogenetic analysis shows that RKNDicV1–6 form a distinct cluster with a clade of unclassified dicistro-like viruses, while PCNDicV1 and PCNDicV2 are located in two separate clusters, each grouping with different unclassified dicistro-like viruses (Fig. 8A and Supplementary Fig. 17).

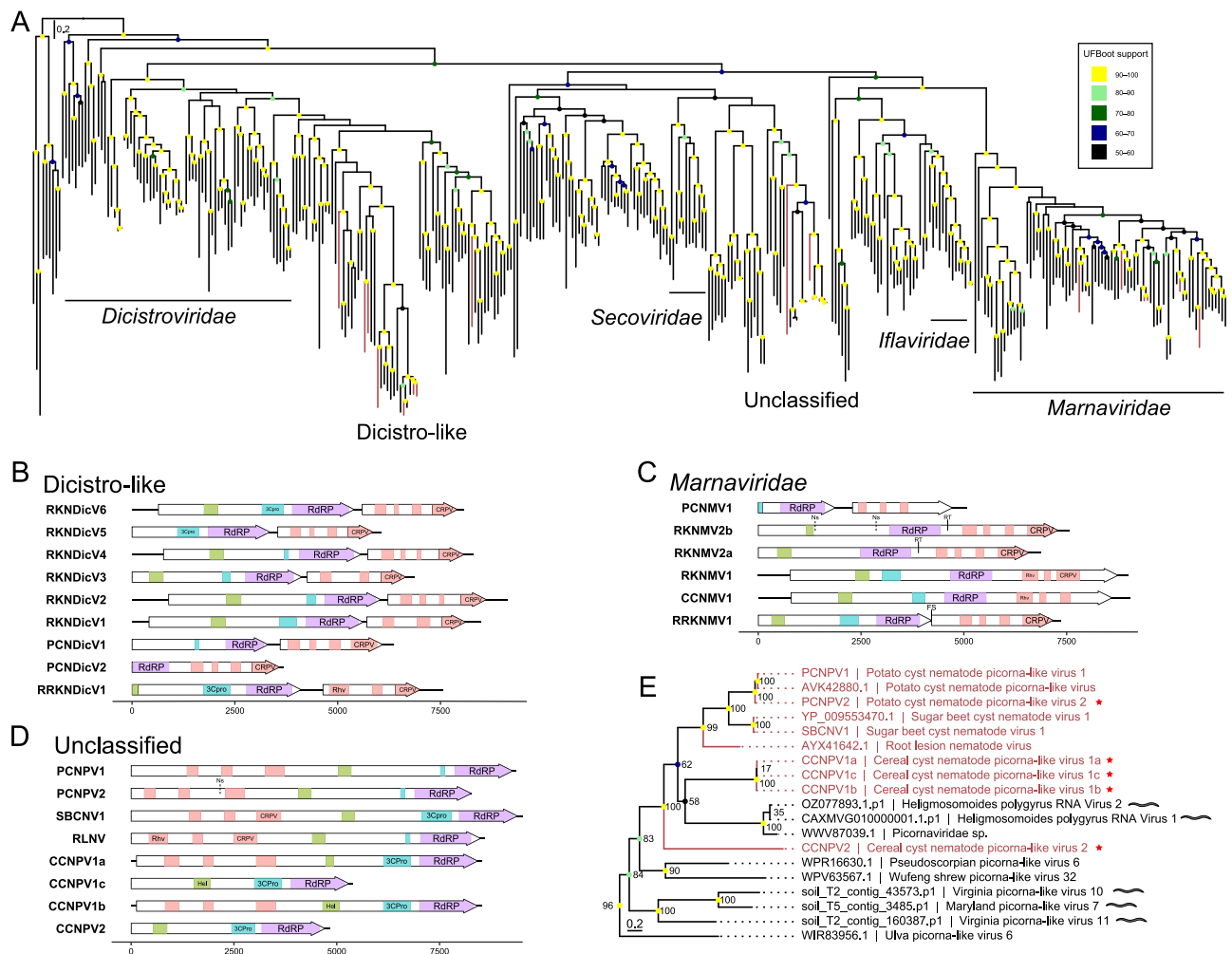


Fig. 8 | Phylogenetic tree and genome organization of PPN-associated viruses belonging to *Picornavirales*. **A** Phylogenetic tree based on the RdRP domain of PPN-associated viruses and their relatives in *Picornavirales*. Q.pfam+F + I + R9 was selected as the best-fit model according to the BIC score. The detailed tree is in Supplementary Fig. 17. **B–D** Genome organization of PPN-associated viruses. Hel, RNA helicase. 3Cpro, cysteine protease. RdRP, RNA-dependant RNA polymerase.

(c) Unclassified *Picornavirales*. We identified eight picorna-like viruses, including five new ones: potato cyst nematode picorna-like virus 2 (PCNPV2), three variants of cereal cyst nematode picorna-like virus 1 (CCNPV1a–c), and cereal cyst nematode picorna-like virus 2 (CCNPV2); as well as three previously reported ones: potato cyst nematode picorna-like virus (PCNPV)²³, sugar beet cyst nematode virus 1 (SBCNV1)²², and root lesion nematode virus (RLNV)²⁴ (Table 1). PCNPV2 shares 82% nucleotide identity and 92% aa identity with the previously reported PCNPV. Phylogenetic analysis based on the RdRP domain shows that PCNPV2, together with all previously identified PPN-infecting picorna-like viruses, forms a monophyletic clade with over 99% UFboot support. The three variants of CCNPV1 share 88–90% nucleotide sequence identity with each other and 41% nucleotide identity with CCNPV2. Both CCNPV1 and CCNPV2 are positioned at the base of PPN-specific picorna-like virus clades (Fig. 8D). Although CCNPV1 and CCNPV2 were only identified in the SRR5942326 run, where 38% of the reads remain unclassified, their close evolutionary relationship to known PPN-infecting picorna-like viruses suggests that their hosts are likely CCNs.

(vii) *Stellavirales*. **(a) *Astroviridae*.** We identified three new astro-like viruses: soybean cyst nematode-associated astro-like virus 1

CRPV, capsid domain (derived from cricket paralysis virus). Rhv, capsid domain (derived from rhinovirus). Ns, site with gaps. RT, stop-codon readthrough.

E Enlarged view of the unclassified clade containing nematode specific picorna-like viruses in Fig. 8A. Animal and soil nematode-associated viruses are followed by a nematode illustration. Newly identified viruses in this study are marked with solid red star.

(SCNastV1), potato cyst nematode astro-like virus 1 (PCNastV1), and potato cyst nematode astro-like virus 2 (PCNastV2). Based on phylogenetic analysis, SCNastV1 clusters with a Ripishyf virus isolated from riverbank sediment, while PCNastV1 and PCNastV2 each form a distinct clade with astro-like viruses, albeit with low UFboot support values (67% and 71%, respectively) (Supplementary Fig. 18). Whether PPNs are the hosts for SCNastV1, PCNastV1, and PCNastV2 remains undetermined (Supplementary Note 1).

(viii) *Bormycovirales*. We identified twelve new ormycoviruses in five types of PPN datasets, including rice root-knot nematode, potato rot nematode, soybean cyst nematode, potato cyst nematode, and reniform nematode. These viruses were designated rice root-knot nematode-associated ormycovirus 1 (RRKNormV1), potato rot nematode-associated ormycovirus 1–4 (PRNORM1–4), soybean cyst nematode-associated ormycovirus 1–4 (SCNORM1–4), potato cyst nematode-associated ormycovirus 1 (PCNORMV1), and reniform nematode-associated ormycovirus 1–2 (RNORMV1–2) (Table 1). Phylogenetic analysis based on RNA1 reveals six distinct evolutionary relationships among these viruses. SCNORMV4 clusters within the *Deltaormycoviridae* family, while SCNORMV2, PCNORMV1, and PRNORMV2 are positioned within the *Alphaormycoviridae* family. The phylogenetic placement of RRKNORMV1 and PRNORMV4 remains

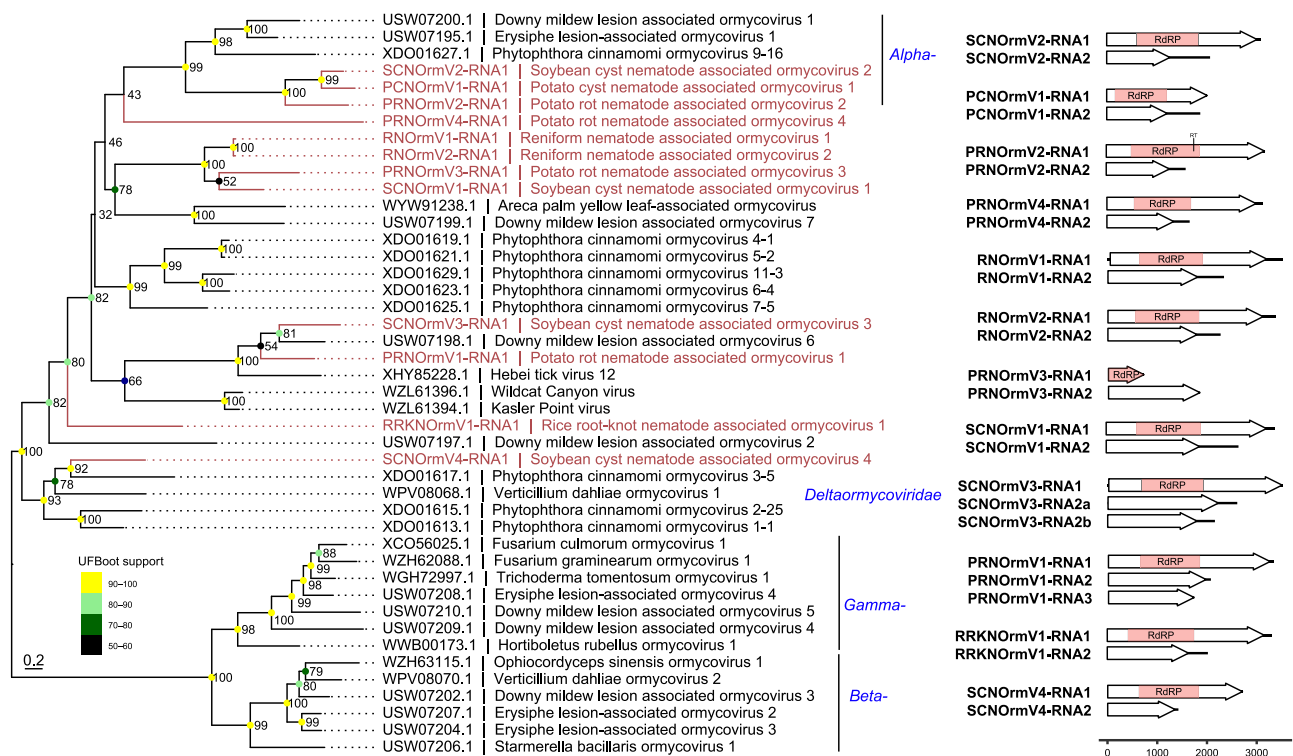


Fig. 9 | Phylogenetic tree and genome organization of PPN-associated viruses in *Bormycovirales*. Phylogenetic tree based on the RdRP domain in RNA1 of viruses in *Bormycovirales*. Q.pfam+F+I+R5 was selected as the best-fit model according to BIC score. RT, stop-codon readthrough.

uncertain due to low UFboot support (<90%). RNormV1–2 and SCNormV1 form a clade, whereas PRNormV1 and SCNormV3 cluster with downy mildew lesion-associated ormycovirus 6 (Fig. 9).

Given that these PPN-associated ormycoviruses are distributed among diverse clades of known fungal or oomycete-associated ormycoviruses, it is possible that some of the identified ormycoviruses originate from contaminants in the library. To address this, we thoroughly scrutinized the taxonomic composition of the libraries, as detailed in the Supplementary Note 1. Our analysis suggests that PRNormV1, RNormV1, and SCNormV1 are highly likely to be associated with their corresponding nematode hosts.

Novel double-stranded RNA viruses

(i) Birnaviridae. Birnaviridae contains bi-segmented dsRNA viruses infecting both vertebrates and invertebrates and displaying a permuted motif (C-A-B motif) in the RdRP domain. We discovered a new birnavirus, tentatively named potato rot nematode associated birnavirus 1 (PRNBiV1), isolated from potato rot nematode populations collected in the field. Segment A of PRNBiV1 contains a large ORF encoding a polyprotein consisting of the capsid protein precursor VP2, endopeptidase VP4, and ribonucleoprotein VP3. Segment B encodes VP1 with the RdRP domain at the N terminus (Supplementary Fig. 19). A BLASTP search shows that VP1 of PRNBiV1 has the strongest match with Lates calcarifer birnavirus (YP_010086266.1), exhibiting 36.4% identity and 77% query coverage. In the VP1-based phylogenetic tree, PRNBiV1 is positioned at the base of all established genera within the family Birnaviridae (Supplementary Fig. 19). The host of PRNBiV1 is likely PRNs, as described in the Supplementary Note 1.

(ii) Durnavirales. (a) Amalgaviridae. We identified two new amalga-like viruses, potato rot nematode-associated amalga-like virus 1 (PRNAmV1) and soybean cyst nematode-associated amalga-like virus 1 (SCNAmV1). Phylogenetic analysis shows that PRNAmV1 and SCNAmV1 are closely related and cluster with Physcomitrium patens amalga virus 1 (Supplementary Fig. 20). The hosts of PRNAmV1 and

SCNAmV1 are likely the plant species co-contaminated in PPN-associated libraries.

Discussion

Through analysis of public SRA data from 25 PPN species and RNA-seq data from field PRN populations, we identified 94 PPN-associated RNA viruses—representing a seven-fold increase over the previously known 12 PPN-viruses. Our findings confirm that PPN-specific viruses form three distinct clades among *Rhabdoviridae*, *Nyamiviridae*, and *Picornavirales*, consistent with earlier research. Notably, we report the first discovery of viruses from the Jingmenvirus group, *Orthomyxoviridae*, *Yueviridae*, and *Bormycovirales* in PPNs, providing new insights into the evolution of these viral lineages. The substantial proportion of viruses identified in field-collected *D. destructor* populations suggests that PPN viral diversity in natural environments likely exceeds current observations.

While viruses in animal-parasitic nematodes and soil nematodes have been previously characterized^{17,18,27,28}, their evolutionary relationships with PPN-associated viruses remained unclear. Our study reveals several evolutionary connections: (1) Three unclassified PPN-associated martellivirals, including BMVV1 which clusters with Brugia malayi RNA virus 1 (from human-parasitic nematodes) in *Martellivirales*; (2) PRNQV1 and PCNQV1 grouping with snake-associated Xinzhou nematode virus 3 in *Qinviridae*; and (3) Seven PPN Jingmen viruses forming a clade with those from animal-parasitic nematodes (*C. oncophora*, *N. brasiliensis*²⁸, and *T. canis*³³). These findings suggest two possible evolutionary scenarios: ancestral infection prior to nematode diversification, or horizontal virus transmission among animal-parasitic, plant-parasitic nematodes, and other organisms.

We identified two novel orthomyxo-like viruses (PCNOV1 and BMOV1) from *Globodera* spp. and *B. mucronatus*, respectively. These viruses possess at least five genomic segments encoding PB1, PB2, GP, PA, and NP, with potential additional segments undetected due to low sequence similarity. Based on viral abundance and SRA taxonomic composition, we propose these nematodes as likely true hosts, thereby expanding the known host range of *Orthomyxoviridae*³⁵. Phylogenetically, these viruses form a distinct clade with Bombus-associated virus Orth1, Wenling orthomyxo-like

virus 2³⁰, and Guangxi sediment orthomyxo-like virus³⁶—suggesting these may also originate from overlooked nematodes in their respective samples.

RNA splicing, a crucial post-transcriptional process for mRNA maturation, is rarely observed in RNA viruses, with known examples limited to influenza viruses³⁷, *Culex tritaeniorhynchus* rhabdovirus³⁸, and Borna disease virus³⁹. We discovered a novel clade of SCN yue-like viruses (SCNYV1a, SCNYV1b, SCNYV2, and SCNYV3) harboring 2–10 introns in their RNA1 and RNA2 segments. The differential intron distribution patterns between RNA1 of SCNYV1a and SCNYV1b suggest rapid evolutionary dynamics. The presence of GU/AU motif at the splice sites suggests that these viruses may utilize the host's RNA splicing machinery for viral protein production (e.g., RdRP).

A key limitation of this study lies in the uncertainty of host assignment for some novel viruses. Defining the hosts of RNA viruses identified from metatranscriptomic data or public SRA RNA-seq data with mixed organisms remains challenging⁴⁰. Although host inference based on sequence similarity and phylogenetic analysis is feasible for characterized viruses, the scarcity of nematode virus-host reference data and the low similarity of most identified viruses complicate confident host determination⁴¹. As indicated by taxonomic analysis and previous literature^{10,42}, contaminated organisms (e.g., plants, fungi, and bacteria) sometimes co-occurs with PPNs, highlighting the necessity of systematic taxonomic/contamination analysis of the SRA runs. Additionally, some viruses may be transmitted by PPNs^{43,44}. For instance, we detected PotVS in two *Globodera* sp., as well as Citrus yellow vein clearing virus and Duck astrovirus in *Bursaphelenchus xylophilus*, suggesting these PPNs might play a role in the transmission of these viruses. Moreover, to confirm the host specificity of the PPN-associated viruses identified in this study, further experimental validation, such as in situ hybridization and infectivity assays, will be necessary in future work.

Approximately one-third (25/94) of the newly identified PPN viruses or PPN-associated viruses in this study were detected in field-collected PRN populations. Most novel bunyaviruses identified in field samples form distinct clades from known viruses (SCNUUKV and SCNRSV), suggesting PPN viral diversity is substantially underestimated, as previous studies were largely confined to laboratory-maintained PPN populations. This finding is consistent with a soil nematode virome study, which has reported that up to 93% (139/150) novel viruses are novel, although some may infect other soil-inhabiting organisms²⁷. However, several limitations are present in both field-collected PRN samples and the public RNA-seq data associated with PPNs. Currently available PPN-related RNA-seq data in SRA databases are heavily biased and homogenized, with many data originating from a few species such as *B. xylophilus* and *G. pallida*. This imbalance undermines meaningful comparative analysis of viral diversity across different nematode species. Although our limited field sampling led to the discovery of numerous previously unknown PRN-associated RNA viruses, broader spatio-temporal sampling and more sequencing efforts will be necessary to fully elucidate the virome of plant-parasitic nematodes.

In summary, our study highlights gaps in understanding virus infecting the most abundant organisms on Earth. Our findings contribute to explore molecular interactions between viruses and PPNs. Expanded investigation of viral diversity will further illuminate evolutionary and ecological dynamics in PPN-virus systems.

Methods

Potato rot nematode sample collection

For virus mining, 11 populations of potato rot nematode were used, including 10 field populations collected from diseased sweet potatoes in four distinct locations in Lulong County, Qinhuangdao City, Hebei Province, China (Supplementary Fig. 1 and Supplementary Table 5), as well as one laboratory-cultured population. For each field population, nematodes were purified via sucrose centrifugation. First, diseased sweet potatoes were cut into pieces and placed into 90-mm-diameter glass dishes. Then, approximately 80 mL of ddH₂O was added to cover the sweet potato pieces. After soaking for 3–4 hours, the pieces were removed, and 0.8 mL of 1% Triton-X 100 solution

was added to each dish to prevent the nematodes from adhering to the glass walls. The solution was poured into a 100 mL centrifuge tube and centrifuged at 4000 × g for 5 minutes. The resulting nematode pellet was resuspended in ddH₂O and transferred to a 1.5 mL centrifuge tube. Following another centrifugation at 4000 × g for 5 minutes, the supernatant was discarded, and the pellet was resuspended in 1 mL of 35% sucrose solution. This mixture was then centrifuged at 2500 × g for 3 minutes. Immediately after this last centrifugation, the supernatant containing the nematodes was transferred into 1000 mL of distilled water to mitigate osmotic pressure-induced mortality. Subsequently, the diluted nematode solution was filtered through a 3-μm membrane. The nematodes retained on the membrane were washed off using ddH₂O. The purified nematodes were washed twice with ddH₂O, flash-frozen in liquid nitrogen, and stored at -80 °C until RNA extraction was performed.

Library preparation and sequencing

Total RNA was extracted from frozen potato rot nematodes using the TransZol Up Plus RNA Kit (TransGen Biotech, China) following the manufacturer's instructions. Total RNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and stored at -80 °C until further processing. To enrich for viral sequences, particularly those lacking poly(A) tails, ribosomal RNA was depleted from the RNA samples using the Globin-Zero Gold rRNA Removal Kit (Illumina, CA, USA). Sequencing libraries were prepared following the manufacturer's protocols. Paired-end sequencing (150 bp) was conducted on an Illumina HiSeq 4000 platform (Illumina, CA, USA). The raw data has been deposited under the NCBI BioProject PRJNA1107688.

Identification of viral sequences

To further investigate the diversity of RNA viruses associated with PPNs, we conducted virus mining of the publicly available transcriptome data from the Sequence Read Archive (SRA) database. Initially, the species names of 25 PPNs were used as keywords to search against the SRA database, and only RNA-seq data were selected for analysis. A total of 536 SRA runs (up to March 30, 2022) were downloaded using the prefetch tool and converted to FASTQ format using the fastq-dump tool. Low-quality reads were removed using the Trimmomatic program (version 0.36) with default parameter settings⁴⁵. The clean reads were then assembled de novo using MEGAHIT (v1.2.9)⁴⁶. Contigs generated from the same nematode species were combined and translated into protein sequences using TransDecoder.LongOrfs with the parameters “-m 100 -G Universal” in TransDecoder (v5.7.1). The resulting protein sequences were annotated using MMseqs2 (version 13.45111) against the clustered NR database. Viral contigs larger than 1 kb were filtered and manually verified. To ensure the absence of host contamination, the viral contigs were aligned against the NT database using megablast within BLAST program. The library information and identified viruses of all SRA runs can be found in Supplementary Table 3.

Viral genome extension and annotation

To recover longer viral genomes, viral contigs sharing the same match in the previous MMseqs2 annotation step were searched against all assembled contigs in the same SRA runs using megablast within the BLAST program, and all the matches were further assembled using the cap3 program⁴⁷. For cloning of full-length viral sequences, a PC3-T7 loop adapter was ligated to the RNA termini using T4 RNA ligase (TaKaRa) at 16 °C. The ligated products were reverse-transcribed into cDNA using M-MLV reverse transcriptase, and the terminal regions were amplified using Taq DNA polymerase (Yeasen Biotechnology Co., Ltd.) with the PC2 primer and virus-specific primers. The resulting PCR products were purified, cloned, and subjected to Sanger sequencing. All the primers used in this study are listed in Supplementary Table 6.

Open reading frames (ORFs) within the viral genomes were predicted using TransDecoder.LongOrfs in TransDecoder (v5.7.1), and further verified using SnapGene (version 6.0). Conserved domains in the predicted proteins were identified using Motif search or HHpred⁴⁸ against the Pfam-A_v37 database. The signal peptide and transmembrane regions of viral

proteins were predicted using SignalP-6.0 and CCTOP server, respectively^{49,50}. Finally, the viral genomes were visualized using the R package gggenes (version 0.5.1).

Viral abundance estimation

Viral contig read counts were estimated using CoverM v0.7.0 (<https://github.com/wwood/CoverM>) in contig mode, with alignments performed using BWA (version 0.7.18). The viral read counts were normalized by the library size and genome length according to the following formula:

$$\frac{\text{mapped reads}}{\left(\frac{\text{total reads}}{10^6}\right) * \left(\frac{\text{length}}{10^3}\right)} = \frac{\text{mapped reads}}{\text{total reads}} * \frac{10^9}{\text{length}} \quad (1)$$

Because of the inherent heterogeneity of SRA datasets, which complicates direct comparisons of viral abundance across different datasets⁵¹, viral abundance estimators were only used as assisting methods to distinguish potential contaminant viruses. As an alternative approach, the k-mer coverage calculated by Megahit was used as a preliminary viral abundance estimator during the initial manual viral filtering step. HISAT2 (v2.2.1) was used to map reads to the genome of four intron-bearing yue-like viruses⁵².

Taxonomic analysis of the downloaded SRA data

Since the downloaded SRA runs originated from different bioprojects with diverse research purposes, the identified viral contigs could potentially have derived from viruses of contaminated organisms such as plants, fungi, or protozoa. To identify the putative hosts of the assembled RNA viruses detected in an SRA run, the taxonomic composition of the organisms within the SRA run was analyzed through taxonomic classification of the raw reads using Centrifuger (v1.0.4-r153)⁵³. The analysis proceeded as follows: First, the preliminary taxonomic composition at the genus level for specific SRA runs exhibiting high candidate virus abundance was inferred based on the taxonomy of matching sequences (with an 85% amino acid identity threshold) of assembled contigs. This step was the same methodology described in the “Identification of viral sequences” section, which was conducted using translated contigs as queries in an MMseqs2 search against the clustered NR database. Subsequently, the representative genomes of species belonging to the genera were retrieved from GenBank and used to construct a custom index using the centrifuger-build tool within Centrifuger⁵³. The raw reads were then classified using the centrifuge, and the classification results were summarized into reports using the centrifuge-kreport tool within Centrifuger⁵³. These reports were converted into a krona-compatible format using kreport2krona.py script⁵⁴, and further visualized using KronaTools (version 2.8.1)⁵⁵. A virus was classified as a contaminant only if it exhibited significant similarity to a known virus, and the host sequences of that known virus were also present in the SRA runs.

Phylogenetic analysis

The RdRP domains of the newly identified viruses were used for phylogenetic analysis. To expedite the multiple sequence alignment of a large number of sequences, the sequences were initially aligned using FAMSA (version 2.2.3-5efa514)⁵⁶. Subsequently, the RdRP domains were extracted and further aligned using Muscle5 (version 5.1) with the -super5 setting⁵⁷. The alignments were manually inspected and refined in Jalview⁵⁸, followed by trimming the ambiguously aligned regions using ClipKIT (version 2.3.0)⁵⁹. The trimmed alignments were subjected to phylogenetic analysis using IQ-TREE (version 2.3.0) with 10,000 ultrafast bootstrap replicates with default settings⁶⁰. The resulting phylogenetic trees were visualized using ggtree (version 3.10.0)⁶¹ and further refined for publication using Adobe Illustrator.

Data availability

The RNA-seq data of potato rot nematode is under the bioproject accession PRJNA1107688. All virus sequences found in field populations of potato rot nematode are available under accession PV640383–PV640423. The viral sequences assembled from public SRA runs are available under accession

BK071874–BK072001. Additional data are available at <https://doi.org/10.5281/zenodo.15617265>.

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Author contributions

X. X. designed and supervised the research. H. M. collected samples and prepared materials for sequencing. H. H. and H. M. wrote the manuscript and performed the bioinformatic analysis. H. H. prepared figures and tables. T. W., J. X., J. Z., J. C., Y. F., Q. C., Y. L., T. C., B. L., X. Y., and D. J. provided suggestions and discussions. T. H. provided English writing suggestions. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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