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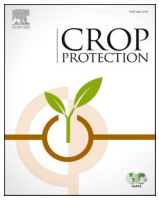

Viral diversity in the European spruce bark beetle *Ips typographus* as revealed through high-throughput sequencing

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ABSTRACT

The European spruce bark beetle, *Ips typographus* is the economically most important biotic damaging agent of Norway spruce. Efforts to delimit beetle populations by trapping, application of chemical insecticides, or mechanically excluding the beetles from their breeding substrates are often expensive and mostly inadequate. The use of natural enemies and viruses is receiving increased research interest as a potential environmentally healthy approach to control pest insect populations, but practical biocontrol methods against *I. typographus* are still lacking. To learn more about putative enemies of this pest species, we used high-throughput sequencing to determine its viral community using beetles collected at a Finnish forest site. The analysis revealed a diverse community of RNA viruses associated with *I. typographus*, including novel viruses that could be affiliated with the classified families *Benyviridae, Metaviridae, Narnaviridae, Partitiviridae, Phenuiviridae, Solemoviridae, Virgaviridae, Tombusviridae*, and proposed family Spiciviridae, as well as unclassified "quenyaviruses". Based on phylogenetic analysis, the viruses were distinct from, but resembled, unclassified viruses originating from other arthropods, and many of them were distantly related to previously described viruses. The possibility that the viruses could be hosted by other organisms than the beetle itself (associated fungi, nematodes and protozoa) was addressed by bioinformatic and phylogenetic analyses and is discussed.

1. Introduction

The European spruce bark beetle, *Ips typographus* L. (Coleoptera: Curculionidae, Scolytinae), is the economically most important biotic damaging agent of Norway spruce, *Picea abies* Karst. (L.). In recent years, Central Europe has suffered from an unprecedented bark beetle calamity. Climate change has strongly contributed to shifting disturbance regimes, including the occurrence of drought periods and bark beetle outbreaks, and the impacts of climate change can be expected to increase in the coming decades (Seidl et al., 2017). Several consecutive hot and dry summers in Europe have severely weakened host trees (Schuldt et al., 2020) and triggered massive outbreaks of *I. typographus* resulting in large-scale tree mortality that has had both economic and social impacts (Hlásny et al., 2021; Senf and Seidl, 2021). In the Nordic countries, the situation is less dramatic although extensive tree mortality caused by *I. typographus* has occurred in the past (Bakke, 1989). However, after the warm and dry summer of 2018 in southern Sweden, the volume of timber killed by *I. typographus* has risen steeply compared to previous outbreaks and corresponds now almost to a quarter of the total annual forest growth (Schroeder and Kärvemo, 2022). Volume of loggings due to bark beetles have increased also in Finland (Ylioja et al., 2023).

Population dynamics of *I. typographus* are characterized by endemic and eruptive states and are strongly influenced by availability of suitable host tree resources. At low population levels *I. typographus* reproduces in weakly-defended, dying spruce trees, and typically abiotic disturbance, e.g. storms, provide wind-felled trees for beetle reproduction (Schroeder, 2001; Komonen et al., 2011; Kärvemo et al., 2014). Trees suffering from heat and drought stress have lowered defenses and are thus vulnerable to bark beetle colonization (Netherer et al., 2021). If there is suddenly a surplus of weakly-defended host trees for breeding *I. typographus* populations can surpass a critical threshold and colonize living, well-defended trees through pheromone-guided mass attacks (Økland and Berryman, 2004; Marini et al., 2013). In spruce-dominated landscapes, high beetle populations are more easily maintained when warm weather accelerates the beetles' development rate and increases the number of generations and sister broods (i.e., a second brood of offspring produced by re-emerging females without the need to mate again) per season (Annila, 1969; Jönsson et al., 2011).

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Management of *I. typographus* outbreaks is challenging especially if populations have reached high densities. Efforts to reduce beetle populations using pheromone traps or trap logs are often expensive and mostly inadequate. Protecting logs from bark beetle attacks by application of insecticides, biocides, watering or physical covering also have limited effect on reducing beetle reproduction. Thus, the aim of management is usually to limit population growth and further attacks on healthy trees by removing weakened and damaged trees and timber from the forest and temporary storage sites before the life cycle of the beetle is completed. Also inter- and intraspecific competition decrease population growth rates and regulation by natural enemies may have more importance at endemic state or in the end of eruption phase when the host resources become depleted.

There is now increased research interest in the use of natural enemies and viruses as an environmentally sound approach to control pest insect populations (Mann and Davis, 2021; Wagemans et al., 2022; Wegensteiner et al., 2015a). Natural enemies of *I. typographus* include woodpeckers, predatory beetles and flies, parasitic wasps and fungi (Wermelinger et al., 2012; Pelto-Arvo, 2020), as well as nematodes and protozoa (Wegensteiner et al., 2015b). Viruses harbored by *I. typographus* are less well studied. However, it has been known for several decades that the spruce bark beetle hosts large DNA entomopoxviruses that are visible with microscopic inspection (e.g., Vanická et al., 2020). Entomopoxviruses appear to be more common in natural forests than in commercial forests, probably because there is a constant supply of suitable breeding substrate for the beetles in natural forests and the populations are more continuous. Unfortunately, contrary to many other entomopoxviruses infecting pest insects of crop plants (Wagemans et al., 2022), the entomopoxviruses of *I. typographus* do not seem very effective in regulating their host populations as the viruses typically have low prevalence (Burjanadze and Goginashvili, 2009; Vanická et al., 2020) and virus-infected adult beetles appear healthy (Wegensteiner and Weiser, 1995). Virus species that do have a significant impact on regulating the populations of pest insects of forest trees include, for example, the nuclear polyhedrosis virus (a member of family *Baculoviridae*) of the European pine sawfly (*Neodiprion sertifer*) and baculoviruses which increase the mortality of the spongy moth (*Lymantria dispar*) (Cory and Myers, 2003; Wagemans et al., 2022).

However, bark beetles in boreal forests have not been systematically studied with methods that would reveal the diversity of their entire virus community. In recent years, high-throughput sequencing (HTS) has provided a completely new approach to virus research, and hence the understanding on virus diversity and host ranges has undergone a revolution. The groundbreaking study by Shi et al. (2016) changed the perception of viral diversity in insects and forms a background for RNA virus detection in arthropods. Despite the ecological and economic importance of *I. typographus*, its virome has remained mostly uncharacterized. Paraskevopoulou et al. (2021) studied the diversity of *Flaviviridae* within an insect transcriptome collection including 1243 insect species. They identified the first two RNA viruses infecting *I. typographus*, i.e. two tombus-like viruses named Coleopteran tombus-related virus OKIAV419 and Coleopteran aspovirus OKIAV413, both from Germany. In this study, we used RNA-sequencing of total RNA to analyze the virome of *I. typographus*, and report for the first time the occurrence of diverse RNA viruses affiliated with members of ten different virus families in the *I. typographus* holobiont, including positive-sense and negative-sense single-stranded RNA (ssRNA) viruses, double-stranded RNA (dsRNA) viruses and virus-like retroelements integrated in the host genome.

2. Materials and methods

2.1. Insects, study site, and HTS

The sampling site was a mature, spruce-dominated stand in Ylöjärvi, southern Finland (about 61°40′ N, 23°35′ E), with the dominating spruces slightly over 100 years old. Although the site was a production forest, no cutting had taken place for at least about 40 years. The sampling site was selected based on the occurrence of periodic *I. typographus* damages since the early 2000's and abundant presence of recently dead Norway spruce trees, which was assumed to have allowed accumulation of viruses in the beetle population. The insects were collected alive from the sampling site and brought to the laboratory where they were quickly frozen to −80 °C. They were stored deep frozen until used for RNA extraction. Ten insects were prepared for total RNA extraction as follows: beetles were rinsed in absolute ethanol and nuclease-free water, the elytra, wings and cuticular membrane were removed and gut (mid- and hindgut) dissected. The guts and tissue fluids were collected and pooled, and total RNA extracted using Spectrum Plant Total RNA Kit (Sigma-Aldrich) following Sutela et al. (2020). Four micrograms of RNA was sent to Macrogen Europe. The TruSeq Stranded Total RNA kit with Ribo-Zero H/M/R Gold (Illumina) was utilized in library preparation for Illumina platform generating paired-end 101 bp raw reads.

2.2. Bioinformatics for virus and ORFan discovery

The raw reads produced from RNA-Seq analysis were de novo assembled using Trinity 2.13.2 (Grabherr et al., 2011) with Trimmomatic plugin (Bolger et al., 2014). *I. typographus* derived contigs were detected using a Blastn run (e-value 10e-6) against *I. typographus* genome assembly (CZU_Ityp_1.0) (Powell et al., 2021). Subsequently, the contigs were omitted resulting in a 7787 non-host contigs of which sequences less than 500 nt were filtered out. Blastx searches (e-value 10e-5) against custom viral protein database with selected DNA, RNA and unclassified viral protein sequences were conducted using the original Trinity assembly as well as with the host- and size-filtered contig sets. The Trinity contig set devoid of host-derived and short (<500 nt) contig sequences was run with Blastx (e-value 10e-6) against the non-redundant (nr) database of National Center for Biotechnology Information (NCBI). Contigs showing no significant similarity with the nr proteins were selected and open reading frames of ≥500 nt translated and considered as ORFan (i.e., non-host RNAs with no similarity to known sequences) if subsequent examination with Blastp (with default settings) did not result in any significant hit.

2.3. Determination of virus origin

In order to examine whether the RNA library contained other potential hosts for viruses than the bark beetles, the *I. typographus*-cleaned and size-filtered Trinity assembly was examined using Blastx (e-value 10e-6) against the nr database of the NCBI and taxonomical records were retrieved from NCBI using the efetch command. In addition, mapping of raw reads was used to examine the possible presence of selected associate organisms of *I. typographus* in the samples. Read mapping was conducted for the following taxa: nematodes of genera *Parasitorhabditis*, *Bursaphelenchus*, *Contortylenchus* and *Cryptaphelenchus,* and protozoa of genera *Gregarina* and *Mattesia*. Table S1 lists the GenBank accessions of sequences used in the mapping (mostly rDNA sequences). As a control, read mapping was also conducted against *I. typographus* 18S rRNA (note that although the total RNA was ribo-depleted, some ribosomal RNA remained in the sample).

The possibility of viral integration into the host genome was examined in cases where related viral taxa are inherently integrated (species of *Metaviridae*) or known to have the potential to integrate into insect genomes (putative members of *Partitiviridae* and *Virgaviridae*). PCR was used to examine whether viral amplification products could be obtained from both non-reverse-transcribed nucleic acid samples (indicating that the virus genome is present as DNA) and reverse transcribed samples (virus genome present as RNA). *I. typographus* total RNAs (~1–2 μg) were converted to cDNA using RevertAid Reverse Trancriptase (Thermo Fisher Scientific) and random hexamer primers as recommend by the manufacturer except for a 5 min denaturation step at 99 °C. DreamTaq

DNA Polymerase (Thermo Fisher Scientific) was utilized in the PCRs with virus-specific primers (Table S2) with cDNA as well as non-reverse-transcribed nucleic acid samples (at least two replicates per sample). The amplicons were either purified using treatment with Exonuclease I (Thermo Fisher Scientific) and rAPid Alkaline Phosphatase (Roche) or NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel) prior Sanger sequencing at Macrogen Europe.

3. Results

3.1. RNA-Seq library: yield, host filtering and search for ORFan sequences

The RNA-Seq analysis yielded 102.9 M reads accessible with BioProject ID PRJNA1007456 at NCBI (https://www.ncbi.nlm.nih.gov/). Trinity de novo assembly produced 74 600 contigs of which almost 90% originated from *I. typographus* based on Blastn search. Contigs showing similarity with *I. typographus* and/or being shorter than 500 nt were filtered out generating a set of 924 contigs which was used in the identification of putative viral contigs, ORFan contigs as well as in the taxonomical examination of putative hosts. The contig set included 115 contigs, encoding for predicted proteins (of 166–1105 aa in length) with no homology with known viruses or cellular organisms in the NCBI GenBank database (Table S3). Similarly, ORFan sequences with no sequence homology have been found in other insect species (e.g., Chiapello et al., 2021). Whether or not these sequences represent true replicating viruses requires further molecular characterization and is beyond the scope of this study.

3.2. Phylogenetic analysis of putative viruses

According to the Blastx analysis against a custom virus database, 268 out of the 74 600 Trinity-produced contigs were identified to share sequence similarities with viruses. After examining these contigs by Blastn to remove host-derived reads not identified during the initial host cleaning step (N = 68), followed by identification of multiple contigs representing the same virus and compiling them, and finally extending the contigs by read mapping, we could identify nineteen different viruses representing ten families and ten orders and with diverse genome organizations. They included viruses with segmented and non-segmented, ssRNA (+), ssRNA (−) and dsRNA genomes, as well as genome-integrated viral elements as described in detail below. The compiled sequences are reported as metagenome-assembled genome (MAG) sequences (a close representation to an actual individual genome) submitted to GenBank with the accession numbers reported in Table 1.

3.2.1. Positive-sense RNA viruses

Tombusviridae, Tolivirales. Three distinct tombus-like viruses were identified in the RNA-Seq library and named Ips tombus-like viruses 1, 2 and 3 (Table 1). Based on Blastx analysis, Ips tombus-like virus 1 (2670 nt; Fig. 1) shared ca. 99% aa sequence identity with the Coleopteran tombus-related virus OKIAV419 described earlier by Paraskevopoulou et al. (2021), and which was characterized as a smaller-than-genome viral sequence with two non-overlapping ORFs. The 3' proximal ORF contained the catalytic core domain of RNA-dependent RNA polymerase (RdRP) in the order *Tolivirales* (cd23179; Fig. 1).

Ips tombus-like virus 2 genome (4165 nt) contained two predicted ORFs encoding putative proteins of 992 and 642 aa (Fig. 1). The shorter ORF2, which was located overlappingly downstream of ORF1, included the catalytic core domain of RdRPs (cd23174) and shared 39% aa identity with Dermatophagoides tombus-like virus from the house dust mite (Vidal-Quist et al., 2021; GenBank MW355887) based on Blastx, whereas ORF1 included the Chroparavirus methyltransferase conserved domain (pfam19223).

Finally, Ips tombus-like virus 3 (Table 1) was represented by a partial sequence of 1364 nt that seemingly contained only the 3' proximal part of the virus encoding two putative ORFs, the upstream partial one

Table 1
Viral sequences detected in *Ips typographus* by high-throughput sequence analysis.

Virus name	Original contig name in Trinity asssembly	Segment	GenBank accession	Size (nt)	GC%	Mapping reads (N)	Average depth by read mapping	Pairwise identity of reads	SNPs (N)
Ips virga-like virus 1	TRINITY_DN48996_c0_g1_i1		OR537183	10 999	32.7%	10 698	98.1	99.6%	21
Ips virga-like virus 2	TRINITY_DN32691_c0_g1_i1		OR537184	3841	34.8%	2633	69.2	99.5%	11
Ips tombus-like virus 1	TRINITY_DN62_c0_g1_i1		OR537185	2670	45.5%	78 269	2967.7	98.3%	112
Ips tombus-like virus 2	TRINITY_DN6790_c0_g1_i1		OR537186	4165	49.5%	3162	76.6	99.7%	12
Ips tombus-like virus 3	TRINITY_DN296_c0_g1_i2		OR537211	1364	46.8%	11 300	830.8	97.6%	75
Ips spici-like virus 1	TRINITY_DN1697_c0_g1_i2		OR537187	7295	44.7%	13 349	184.6	98.6%	93
Ips narna-like virus 1	TRINITY_DN3333_c0_g1_i1		OR537188	2983	44.6%	32 140	1084.2	99.3%	21
Ips narna-like virus 2	TRINITY_DN1212_c0_g1_i1		OR537189	3589	44.8%	18 230	510.6	99.1%	45
Ips partiti-like virus 1	TRINITY_DN17762_c0_g1_i1	dsRNA1	OR537190	1802	39.9%	76 533	4203.5	99.4%	8
	TRINITY_DN3677_c0_g4_i2	dsRNA2	OR537191	1422	46.0%	242 237	16 100.7	99.1%	11
	TRINITY_DN3677_c0_g1_i1	dsRNA3	OR537192	1459	43.7%	141 191	9079.2	99.1%	20
	TRINITY_DN9226_c0_g3_i1	dsRNA4	OR537193	1430	41.9%	123 965	8273.8	99.3%	5
Ips sobemo-like virus 1	TRINITY_DN121_c0_g1_i2	RNA1	OR537194	2936	50.5%	84 413	2885.6	98.7%	71
	TRINITY_DN108_c0_g1_i4	RNA2	OR537195	1630	53.7%	32 304	2003.9	98.3%	33
Ips phenui-like virus 1	TRINITY_DN5171_c0_g2_i2	L	OR537196	8331	34.0%	11 770	142.6	99.6%	17
Ips phenuiviral-like M segment 1	TRINITY_DN16811_c0_g1_i1	M	OR537197	5751	33.4%	6773	118.3	99.6%	9
Ips phenui-like virus 2	TRINITY_DN20624_c0_g2_i1	L	OR537198	6709	38.4%	11 624	174.7	99.6%	4
Ips phenuiviral-like M segment 2	TRINITY_DN13852_c0_g1_i1	M	OR537199	4166	33.9%	12 320	298.3	99.6%	3
Ips phenuiviral-like S segment 1	TRINITY_DN61177_c0_g1_i1	S	OR537200	1193	40.7%	17 644	1478.8	99.6%	3
Ips erranti-like virus 1	TRINITY_DN1967_c0_g1_i10	Int.	OR537201	11 548	43.5%	16 784	146.8	98.5%	325
Ips erranti-like virus 2	TRINITY_DN3733_c0_g1_i6	Int.	OR537202	4893	38.6%	6408	128.8	96.4%	348
Ips erranti-like virus 3	TRINITY_DN9101_c0_g1_i4	Int.	OR537203	7863	43.0%	10 086	127.2	87.4%	204
Ips erranti-like virus 4	TRINITY_DN2441_c0_g1_i1	Int.	OR537204	3737	45.0%	3899	103.4	97.1%	167
Ips erranti-like virus 5	TRINITY_DN1238_c0_g1_i11	Int.	OR537205	4772	46.2%	2472	52.3	99.1%	72
Ips erranti-like virus 6	TRINITY_DN3231_c0_g1_i1	Int.	OR537206	2829	36.4%	21 797	777.9	99.3%	27
Ips quenya-like virus 1	TRINITY_DN22650_c0_g1_i1	Segment 1	OR537207	1559	42.8%	639	41.3	99.7%	5
	TRINITY_DN14927_c0_g1_i2	Segment 4	OR537208	1538	40.4%	819	52.0	99.3%	6
	TRINITY_DN66219_c0_g1_i1	Segment 5	OR537209	1142	42.5%	380	33.6	99.6%	4
Ips beny-like virus 1	TRINITY_DN48597_c0_g1_i1		OR537210	2562	44.6%	1066	42.0	97.8%	102

(ORF2) resembling hypothetical protein 2 of Soybean thrips tombus-like virus 4 (Thekke-Veetil et al., 2020) with 58% Blastp identity and affinities to viral coat proteins (pfam00729). The predicted ORF1 protein did not match any sequences available in GenBank. The RdRP-encoding sequence could not be derived from raw reads thus preventing more thorough phylogenetic analysis for Ips tombus-like virus 3.

Compiling the Trinity contigs and mapping of the raw reads against the viral sequences revealed that Ips tombus-like virus 1 exhibited numerous SNPs suggesting that more than one closely similar variant of the virus was present in the RNA-Seq library (possibly three based on SNPs observed at single positions; pairwise identity scores based on read mapping are reported in Table 1). The longest sequence variant was selected as the representative MAG. Similarly, the partial sequence of Ips tombus-like virus 3 contained SNPs suggesting intraspecific variation. In turn, Ips tombus-like virus 2 was almost invariable (without SNPs) and therefore probably originated from a single infected host beetle or represented a single variant.

Based on phylogenetic analysis (Fig. 2), Ips tombus-like virus 1 groups among members of the proposed genus "Aspovirus" (Paraskevopoulou et al., 2021), while Ips tombus-like virus 2 affiliates with members of the proposed "Surivirus" clade. Both taxa group among other insect-derived viruses.

***Sobemovirus, Solemoviridae, Sobelivirales*.** There were two contigs affiliated with sobemoviruses (Table 1). These were concluded to comprise the genome of a bisegmented virus named as Ips sobemo-like virus 1 that resembles earlier described arthropod-derived sobemo-related viruses (Shi et al., 2016, Fig. 3). RNA1 (2936 nt; Table 1) is predicted to encode two overlapping proteins (627 and 521 aa), where the polymerase-encoding region is located downstream of a putative protein of unknown function (no conserved domains were identified by Blastp). The predicted RdRP-encoding ORF (Fig. 1) in RNA1 segment is similar to related viruses and is predicted to start with the alternative initiation codon TTG. TTG is also the start codon used in Mlepnos solemo-like virus strain GUNF_DN26588-21 (QIJ70122.1) originating from the flea species *Spilopsyllus cuniculi* and was somewhat related to Ips sobemo-like virus 1 based on Blastp (35% identity over 98% query cover for the ORF2-encoded putative RdRP). RNA2 (1630 nt) has two predicted ORFs in the same reading frame, but based on closer examination of related viruses, for example Wuhan house centipede virus 5 strain WHYY23902 segment 2 (GenBank: KX882922.1; Shi et al., 2016) they could represent a single ORF with a readthrough amber stop codon located in nt position 771–773 and encoding a putative capsid protein of 462 aa (Fig. 1).

Based on read mapping, there were SNPs present in both genomic RNAs, and more than two different viral variants are expected to be present in the sample pool, as some variable sites exhibit three different nucleotides.

***Virgaviridae, Martellivirales*.** Two different virga-like viruses were observed in the RNA-Seq library. One contig could be extended to the total length of 10 999 nt and contained two long predicted ORFs as well as three shorter ones. The putative virus was named Ips virga-like virus 1 (Table 1, Fig. 1). ORF1 was predicted to encode a polyprotein with conserved motifs for a helicase and RdRP, and ORF2 included a conserved motif for a glycoprotein (GP). The GP conserved motif is typical of negative-sense viruses such as members of *Bunyavirales/Phasmaviridae* and not found in classified members of *Virgaviridae*. However, Shi et al. (2016) have identified a virus with similar genome organization with a predicted GP motif earlier. This virus, called Hubei virga-like virus 23 (GenBank KX883774), originates from a Chinese mosquito species. The three small ORFs (3–5) did not contain conserved motifs.

The second virga-like contig was partial (3841 nt) and could not be extended by read mapping. It was predicted to encode a polyprotein containing a methyltransferase conserved domain (pfam01660) but lacked most of the predicted RdRP region. It should be noted that various virga/nege-like viruses have been identified to appear as truncated sequences endogenized into insect genomes (Kondo et al., 2019) so there is a possibility that this putative virus, named Ips virga-like virus 2 (Table 1), would be similarly integrated. However, PCR-based analysis suggested it is not integrated as virgavirus amplification products were obtained only from cDNA templates and not from non-reverse-transcribed nucleic acid templates (amplification from the same templates was successful for the integrated errantivirus, see 3.4).

Read mapping revealed very few putative sequence polymorphisms in the virga-like viruses, and all contigs representing each virus species were 100% identical. Based on phylogenetic analysis (Fig. 4), Ips virga-

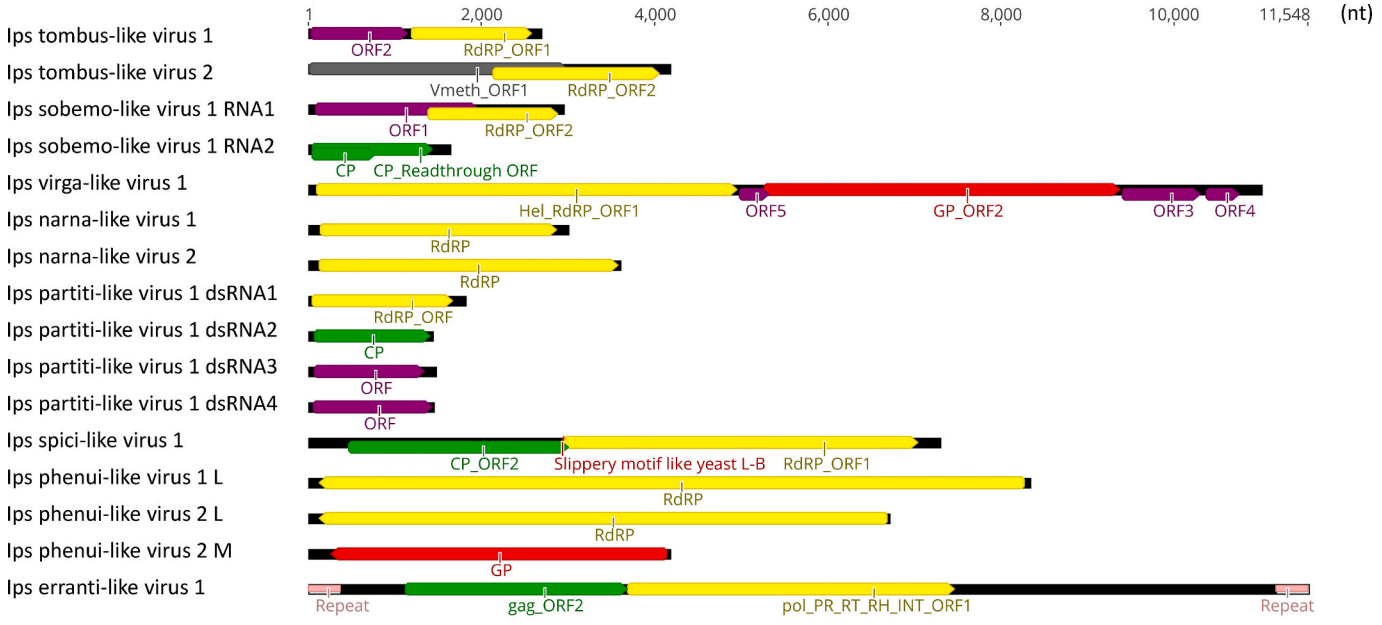
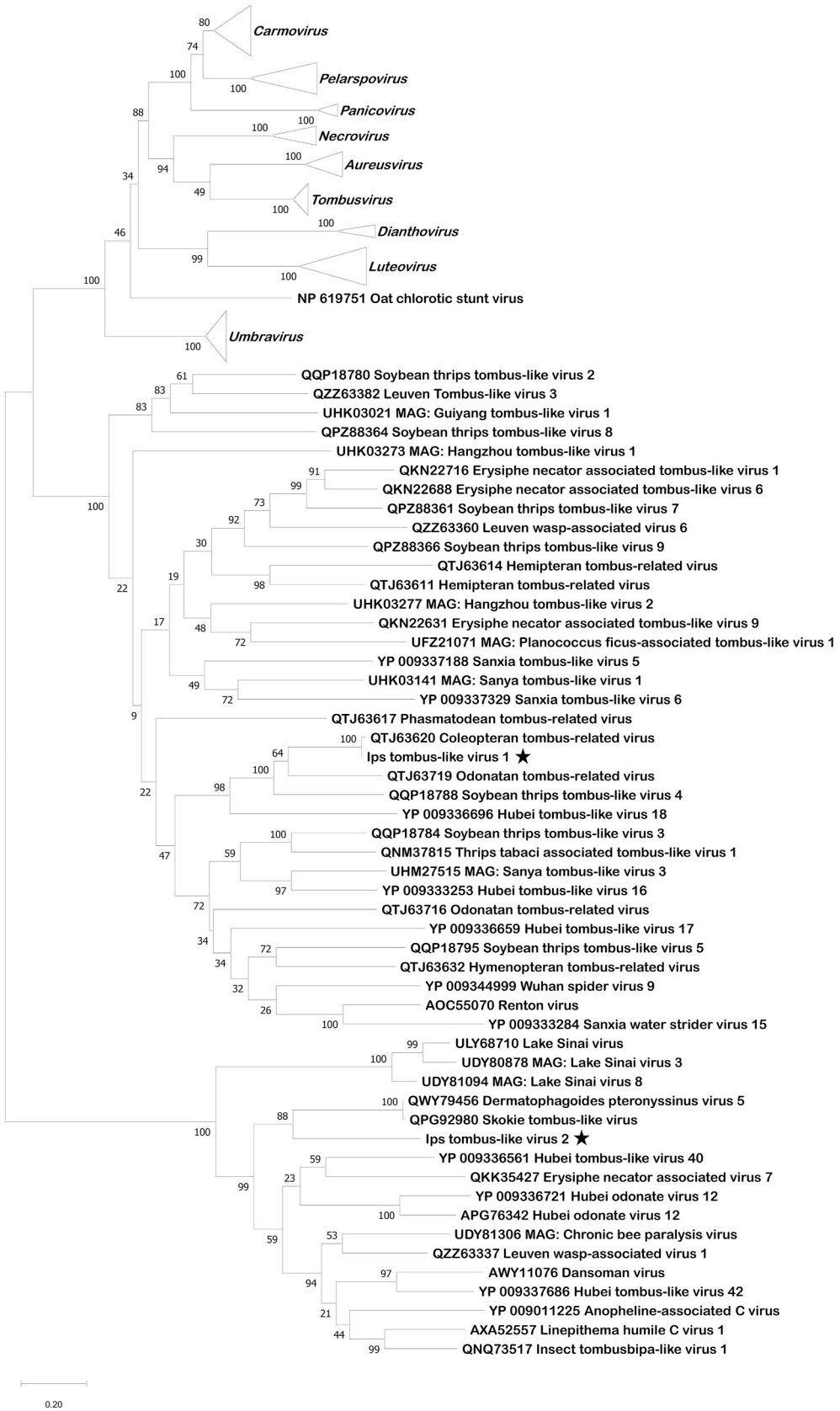


Fig. 1. Schematic illustration of the genome organizations, predicted open reading frames (ORFs) and predicted protein products of virus-like sequences detected in *Ips typographus*. Further partial virus-like sequences are reported in Table 1. Hel = Helicase; RdRP = RNA-dependent RNA polymerase; Vmeth = Viral methyltransferase; CP = capsid protein; GP = glycoprotein; gag = retrotransposon gag or capsid-like protein; pol = polymerase; PR = Retropepsin-like; RT = Reverse transcriptase; RH = RNAseHI; INT = Integrase.



(caption on next page)

Fig. 2. A phylogenetic tree including classified genera of family *Tombusviridae* and related unclassified viruses based on alignment of RdRP aa sequences. The alignment was generated using MAFFT v7.490 and the evolutionary history was inferred by using the Maximum Likelihood method and Le_Gascuel_2008 model with 5 gamma categories (+ *G* + *I*). All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. Evolutionary analyses were conducted in MEGA11 with 500 bootstrap repeats (Tamura et al., 2021). The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Stars denote the putative tombus-like viruses found in *Ips typographus*.

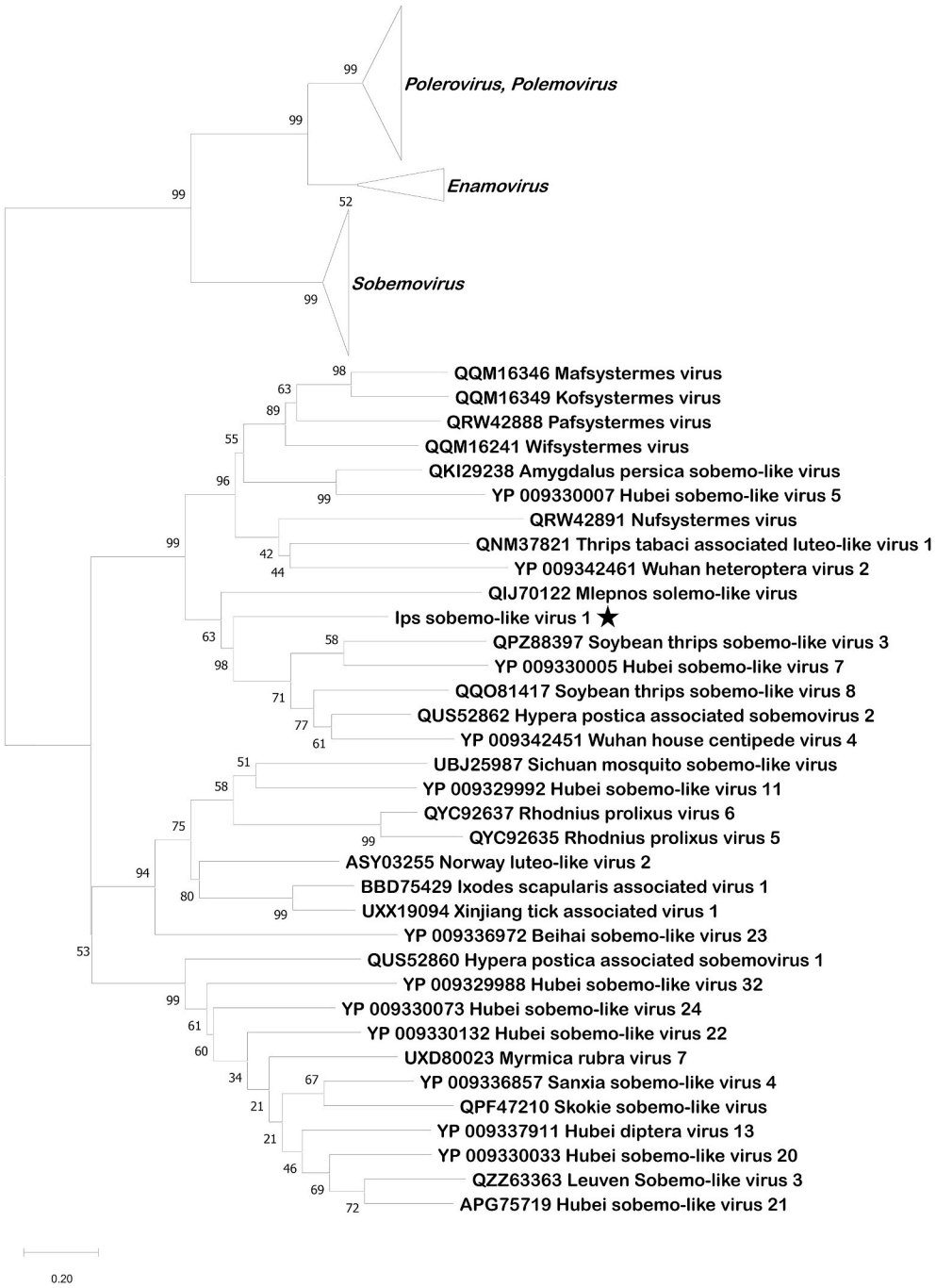


Fig. 3. A phylogenetic tree including classified genera of family *Solemoviridae* and related unclassified viruses based on alignment of RdRP aa sequences as described in Fig. 2. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. A star denotes the putative sobemo-like virus found in *Ips typographus*.

like virus 1 clusters with other insect-derived virga-like viruses but does not affiliate with any particular subclade. All the insect-derived virga-like viruses resembling this virus cluster outside classified genera in *Virgaviridae* (Fig. 4). Based on Blastp analysis of the replicase protein, the closest relative of both Ips virga-like viruses detected in this study was Myzus persicae nege-like virus 1 (MW528419.1) originating from an aphid species. In both cases, sequence similarity was relatively low: 30% over 58% query cover for the RdRP-encoding ORF2 of Ips virga-like

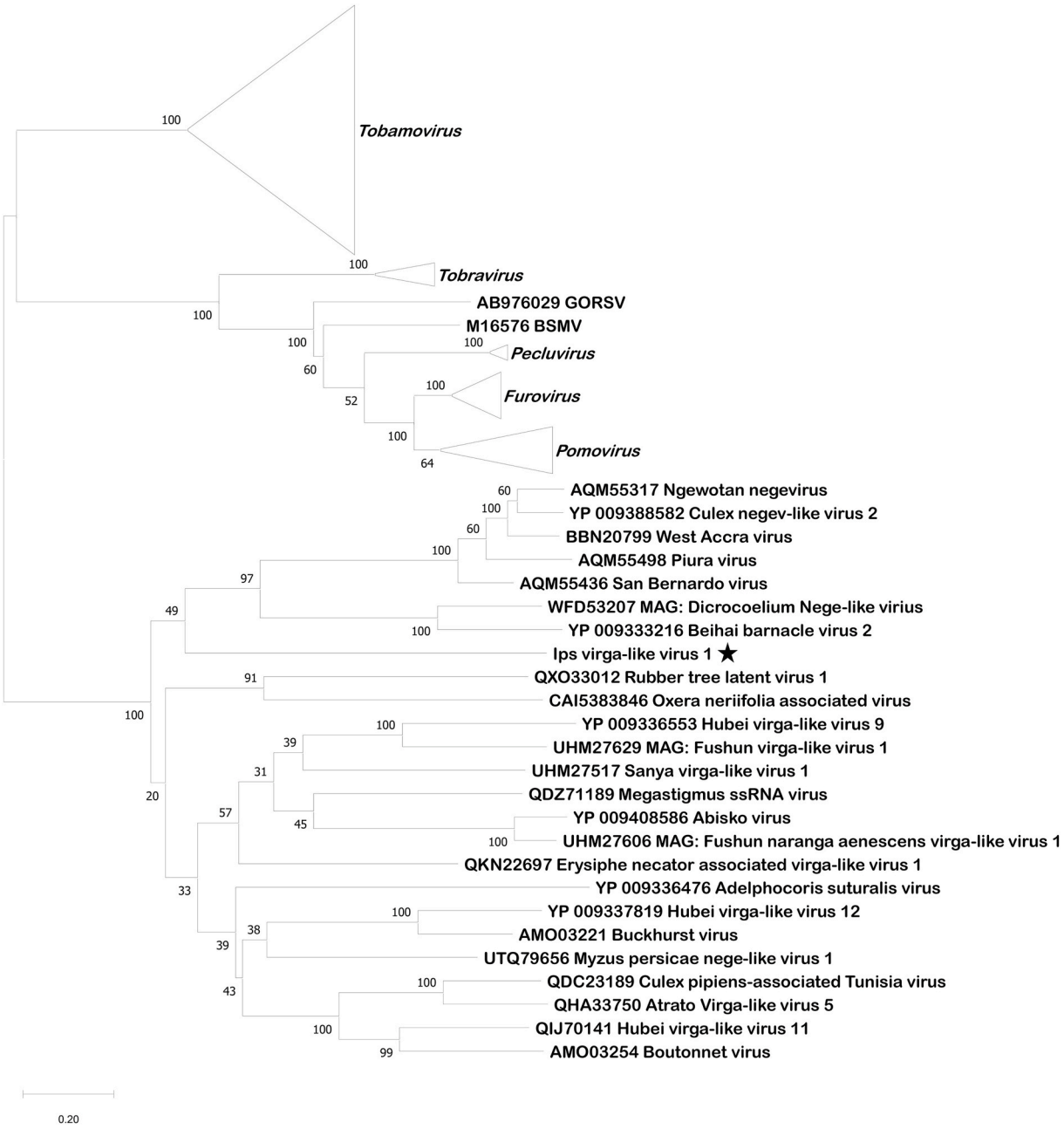


Fig. 4. A phylogenetic tree including classified genera of family *Virgaviridae* and related unclassified viruses based on alignment of RdRP aa sequences as described in Fig. 2. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. A star denotes the putative virga-like virus found in *Ips typographus*.

virus 1 and 26% over 37% query cover for the partial ORF of Ips virga-like virus 2.

Narnaviridae, Wolframvirales. Two distinct viruses resembling members of *Narnaviridae* were observed in the RNA-Seq library (Table 1, Fig. 1). The first one (2983 nt) contained one predicted coding-complete ORF encoding a putative RdRP (908 aa) including the conserved domain for *Narnaviridae* RdRP (cd23177). It was named Ips narna-like virus 1. The second one named Ips narna-like virus 2 (3589 nt) encoded a putative protein of 1153 aa that contained the *Botourmiaviridae* RdRP conserved motif (cd23183). The narna-like viruses found in *I. typographus* did not contain a long negative-sense ORF as found in some members of proposed genus Alphanarnavirus (Dinan et al., 2020; Retallack et al., 2021).

Based on read mapping, Ips narna-like virus 1 is virtually devoid of polymorphic nt sites, and Ips narna-like virus 2 has very few polymorphisms (Table 1). Phylogenetic analysis reveals that Ips narna-like virus 1 resembles most closely the Sherlock virus (Harvey et al., 2019; GenBank QED21500.1) (44% aa identity with 97% query cover) originating from *Stephanocircus harrisoni*, a flea species, as well as other narna-like viruses from invertebrates (Fig. 5). Ips narna-like virus 2 clusters rather distantly with a virus clade containing arthropod, oomycete and fungal narna-like viruses (approximately 30% or less aa identity by Blastp), the closest Blast match being Erysiphe necator associated narnavirus 8 from an ascomycete fungus (GenBank QJT93740.1; 31% aa identity with 77% query cover). Both Ips narna-viruses were tentatively included in the proposed genus "Betanarnavirus" (Fig. 5).

Quenyaviruses (unclassified). Three contigs resembling recently described insect viruses named quenyaviruses were discovered in the RNA-Seq library. These showed resemblance to a partial RdRP-encoding

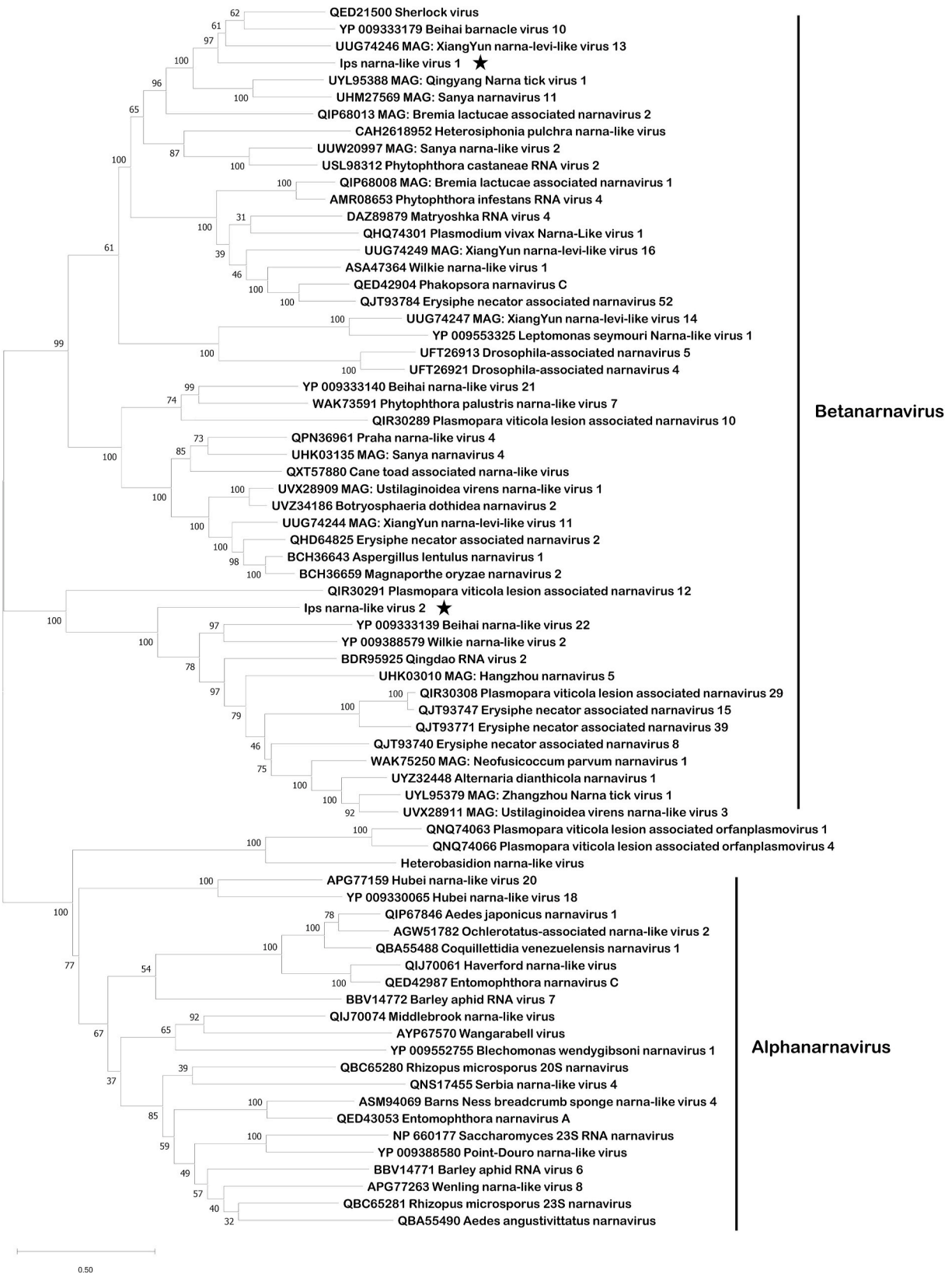


Fig. 5. A phylogenetic tree including classified genera of family *Narnaviridae* and related unclassified viruses based on alignment of RdRP aa sequences as described in Fig. 2. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Stars denotes the putative narna-like viruses found in *Ips typographus*, and proposed genera Alphanarnavirus and Betanarnavirus are indicated.

genome segment 5 (DN66219_c0_g1_i1; 1142 nt, no sequence extension was possible by read mapping), genome segment 1 (1559 nt) with unknown function and genome segment 4 with unknown function (1673 nt) of quenyaviruses (Obbard et al., 2020). The read counts for this virus were very low (Table 1), and we could only determine a partial genome not suitable for reliable phylogenetic analysis, but based on Blastx analysis, the segments were related to a handful of viruses all discovered by metagenomic assembly from arthropods, such as mixed samples of spiders or crickets (Obbard et al., 2020) and the Enontekio quenyavirus from Finnish mosquitoes (Truong Nguyen et al., 2022)(33–34% identity with 78–87% query cover by Blastx for the RdRP encoding segment). "Segment 1" resembled that of the quenyavirus named Bawangfen virus from lizard gut (*Calotes versicolor*) and Nete virus from the moth *Crocallis elinguaria* (Obbard et al., 2020), and "segment 4" resembled a similar sequence in Jiangan, Sina and Nai viruses of crickets or wasps (Obbard et al., 2020).

Benyviridae, Hepelivirales. One virus was affiliated with beny-like viruses. Only a partial sequence representing this virus, named as Ips beny-like virus 1, was obtained after read mapping, and comprised 2562 nt (Table 1). The sequence contained a partial predicted ORF of 807 nt corresponding to the amino-proximal end of a polyprotein of insect beny-like viruses. It contained a conserved motif pfam01443 (Viral Superfamily 1 RNA helicase). However, the catalytic core domain of RdRP in the family *Benyviridae*, which is located in the carboxy-terminal end of insect beny-like virus RdRPs, was lacking from the sequence, as was the downstream sequence encoding a putative capsid protein in insect beny-like viruses with complete nonsegmented genomes of over 5 kb. As the putative polymerase was partial, no thorough phylogenetic analysis was conducted, but the two most closely related viruses according to Blastx analysis were Guiyang benyvirus 1 (64% identity with 94% query cover; GenBank UHK03084) and Diabrotica undecimpunctata virus 2 (61% identity with 94% query cover; QIT20101). The latter one is found in the leaf beetle species *Diabrotica undecimpunctata howardi* (Liu et al., 2020), and the first one from *Harmonia axyridis*, a ladybug.

3.2.2. Double-stranded RNA viruses

Partitiviridae, Durnavirales. There were four distinct contigs related to partitivirid sequences (Table 1, Fig. 1). The longest contig (dsRNA1, 1802 nt) contained a single predicted ORF with an RdRP conserved domain (pfam00680) and was related (65% aa identity and 97% query cover) to the Hubei partiti-like virus 2 (KX884045) originating from a sample including a mixture of different arthropods (Shi et al., 2016). Only one genome segment is reported for Hubei partiti-like virus 2. Three other partiti-like sequences were related to different genome segments of Atrato partiti-like virus 1 from the mosquito species *Mansonia titillans* and Hubei tetragnatha maxillosa virus 8 (from a spider species), both of which have four reported genome segments. Each dsRNA segment has one predicted long ORF, and dsRNA2 (1422 nt) encodes for the predicted coding complete CP (no conserved motifs; 447 aa; 50% identity over 98% query cover by Blastp with the Atrato partiti-like virus 1). The predicted ORF in dsRNA3 (1459 nt and 423 aa) resembles a hypothetical protein of Atrato partiti-like virus 1 with 35% aa identity with 95% query cover, and the putative ORF in dsRNA4 (1430 nt; 456 aa) resembles a hypothetical protein of Hubei tetragnatha maxillosa virus 8 with 37% identity and 93% query cover. Based on phylogenetic analysis (Fig. 6), the Ips partiti-like virus 1 affiliates with the proposed genus "Epsilonpartitivirus" considered in a broad sense (Shi et al., 2016; Nerva et al., 2017; Jiang et al., 2019).

The level of sequence polymorphisms was low in the genome segments of the partitivirus based on read mapping and contig alignment (Table 1). No poly(A) tails typical of some genera of *Partitiviridae* were observed. The two dsRNA elements with ORFs of unknown function showed some characteristics of genome-integrated sequences based on Blastn analysis (homology with host genome sequences). However, PCR-based analysis confirmed that the partitivirus genome segments were only amplifiable from cDNA templates and not non-reverse-transcribed nucleic acid templates, therefore suggesting that the virus was not integrated (amplification from the same templates was successful for the integrated errantivirus, see below).

Spiciviridae, *Ghabrivirales*. One virus resembling members of a newly proposed family "Spiciviridae" was detected in the library (7295 nt; Table 1). A recent proposal for the reorganization of the *Ghabrivirales* order (https://ictv.global/files/proposals/) suggests including similar viruses, formerly designated as "toti-like" viruses, in a new genus named "Spicivirus". The virus detected in our RNA-Seq library was named Ips spici-like virus 1 and contained two partially overlapping OFRs typical of many members of proposed suborder "Alphatotivirineae" (including spicivirids), where the RdRP-encoding ORF1 (4098 nt; 1365 aa) is located downstream of ORF2 (2535 nt; 845 aa) (Fig. 1). In many classified *Ghabrivirales* members, ORF2 encodes the CP, and although Blastp identified no conserved motifs in ORF2, the closest matches were to structural proteins (capsid-like proline-alanine rich proteins) of related viruses, such as Rose latent virus (ON872171) and Circulifer tenellus virus 1 (NC_014360; Spear et al., 2010). Based on Blastp analysis, the virus RdRP was most similar (39% aa identity with 65% query cover) with that of Spissistilus festinus virus 1 (SpFV1; GenBank NC_014359; Spear et al., 2010), a typical member of genus Spicivirus (Fig. 7). As with SpFV1, ORF1 was predicted to be translated utilizing a −1 frameshift compared to ORF2 (ORF2 in +3 frame and ORF1 in +2 frame). The predicted frameshift site contained the slippery motif GGAUUUU that is also found in the yeast L-B virus, which is a totivirus in order *Ghabrivirales* (Bekaert and Rousset, 2005). In Ips spici-like virus 1, the putative slippery motif was located 52 nt upstream of the stop codon of ORF2. Based on read mapping, there were SNPs present in the spicivirus sequence (Table 1), and it appears probable that at least two different variants of the spici-like virus were present in the RNA-Seq library.

3.3. Negative-sense RNA viruses

Phenuiviridae, Bunyavirales. We detected genome segments of at least two different phenui-like viruses in the RNA-Seq library (Table 1; Fig. 1). The longest phenui-like contig (8331 nt) contained one long ORF coding for a putative polyprotein of 2716 aa with conserved motifs for the Bunyavirus RdRP (pfam04196), viral protein of unknown function (pfam12603) and bunyavirus endonuclease domain (pfam15518). This sequence was concluded to correspond to the L segment of a new bunya-like virus named Ips phenui-like virus 1. It was most closely related to the unclassified Hangzhou phenuivirus 2 (UHK03194.1) from *Tetragnatha nitens* (a spider species) (42% aa identity with 51% query cover by Blastp)(Fig. 8). Another phenui-like virus named Ips phenui-like virus 2 (6709 nt) resembled several uukuviruses, e.g., the Huangpi tick virus 2 (NC_031138.1; Li et al., 2015) from *Haemaphysalis* sp. in China (35% aa identity with 83% query cover by Blastp) as well as ixoviruses, such as the Blacklegged tick plebovirus 1 (ANT80544; Cross et al., 2018; 34% aa identity with 81% query cover by Blastp), but clustered outside the classified phenuiviruses (Fig. 8).

We also discovered a contig (4166 nt) that assumably encoded the M segment of a phenui-like virus, including a putative phlebovirus glycoprotein (GP) (conserved motif pfam07245)(Table 1). This contig was most closely related to the Huangpi tick virus 2 (24% aa identity with 50% query cover), and assumably represents the second genome segment of Ips phenui-like virus 2. A fourth contig also contained a putative GP motif (5751 nt). This contig contained a section with low amount of reads and there remains a possibility that the contig is misassembled, but it showed distant relationship with the glycoprotein of the Hymenopteran phasma-related virus OKIAV244 (GenBank QMP82315.1; Käfer et al., 2019; 21% aa identity with 45% query cover). All the phenuivirus-like L and M segment contigs were subjected to read mapping, and there was no further extension to the sequences and virtually no sequence variation (Table 1). Finally, one contig corresponded to phlebovirus S segment with a coding capacity for a

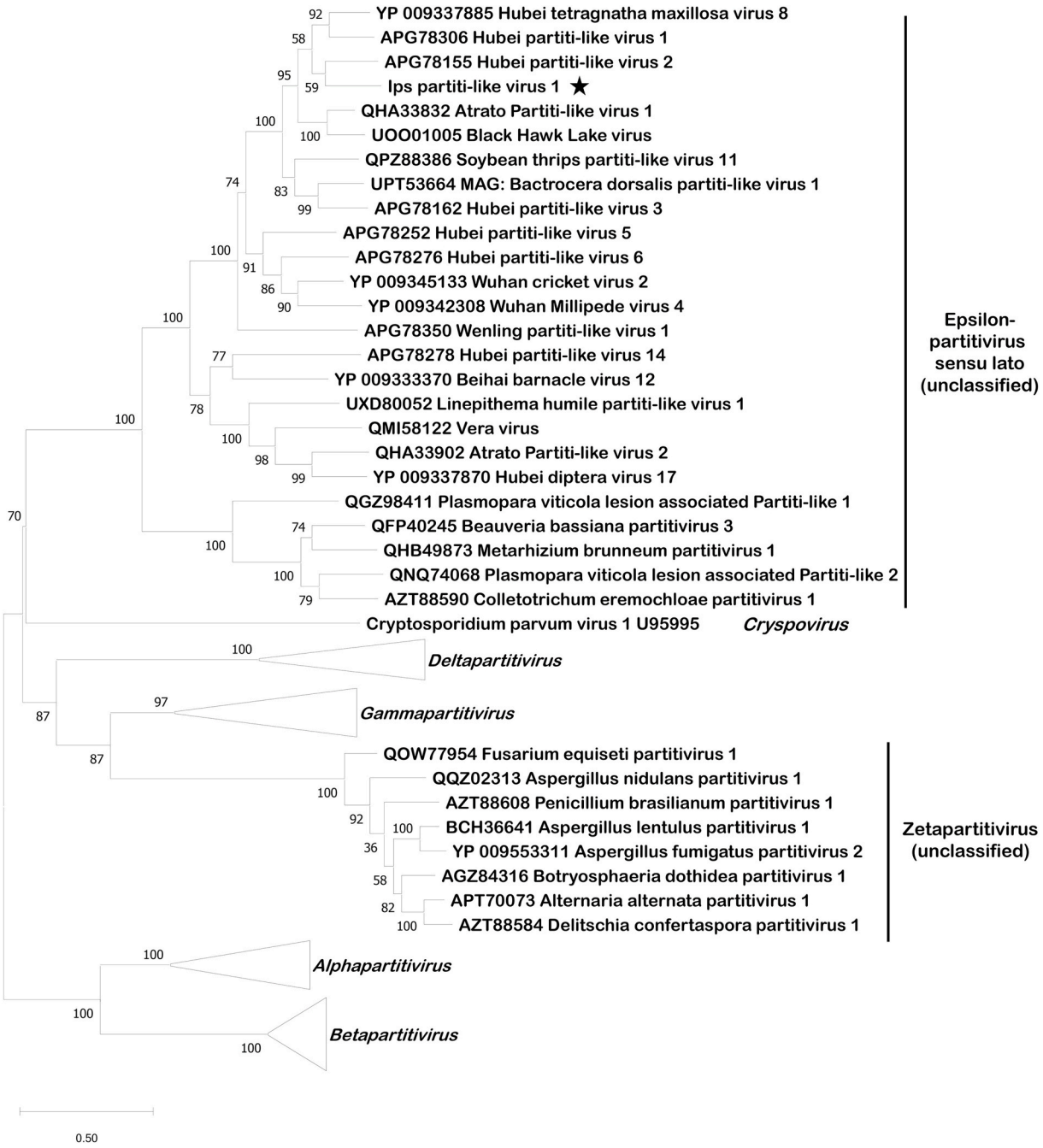


Fig. 6. A phylogenetic tree including classified genera of family *Partitiviridae* and related unclassified viruses based on alignment of RdRP aa sequences as described in Fig. 2. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Proposed genera Epsilonpartitivirus and Zetapartitivirus are indicated. A star denotes the putative partiti-like virus found in *Ips typographus*.

nucleocapsid protein (conserved domain pfam05733 for *Tenui/-Phlebovirus* nucleocapsids). The contig could be extended to a length of 1193 nt, and it shared the closest sequence homology with Qingdao tick uukuvirus (GenBank OQ513656; Hu et al., 2023; 31.05 aa identity with 78% query cover). The level of sequence polymorphisms was very low in all phenui-like viral sequences (Table 1).

3.4. Retrotransposons

Metaviridae, Ortervirales. There were several contigs and at least six distinct sequence types with affinities to retrotransposons in the family *Metaviridae*, genus *Errantivirus* (Table 1). The majority of them were most closely related to the Halyomorpha halys erranti-like virus 1 (URQ09129.1; see Fig. 9), and one (named Ips erranti-like virus 5; DN1238_c0_g1_i11) to the Hemigrapsus takanoi nimavirus (GenBank BDT63269.1; Kawato et al., 2019; Blastx aa identity of 40% with 51% query cover). The longest errantivirus sequence (11 548 nt) contained two predicted ORFs and had tandem repeats of 372 nt at sequence ends (Fig. 1). ORF1 showed coding capacity to a polyprotein containing conserved motifs for a reverse transcriptase (cd01647), RNAseH (cd09274), retropepsin (cd00303), and an integrase (pfam17921 and pfam00665), and shared 30% aa identity with 79% query cover with the Halyomorpha halys erranti-like virus 1 based on Blastp analysis. ORF2 showed affinities to gag, i.e., capsid-like proteins of

Fig. 7. A phylogenetic tree including proposed genera of order *Ghabrivirales* and related unclassified viruses based on alignment of RdRP aa sequences as described in Fig. 2. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. A star denotes the putative spici-like virus found in *Ips typographus*.

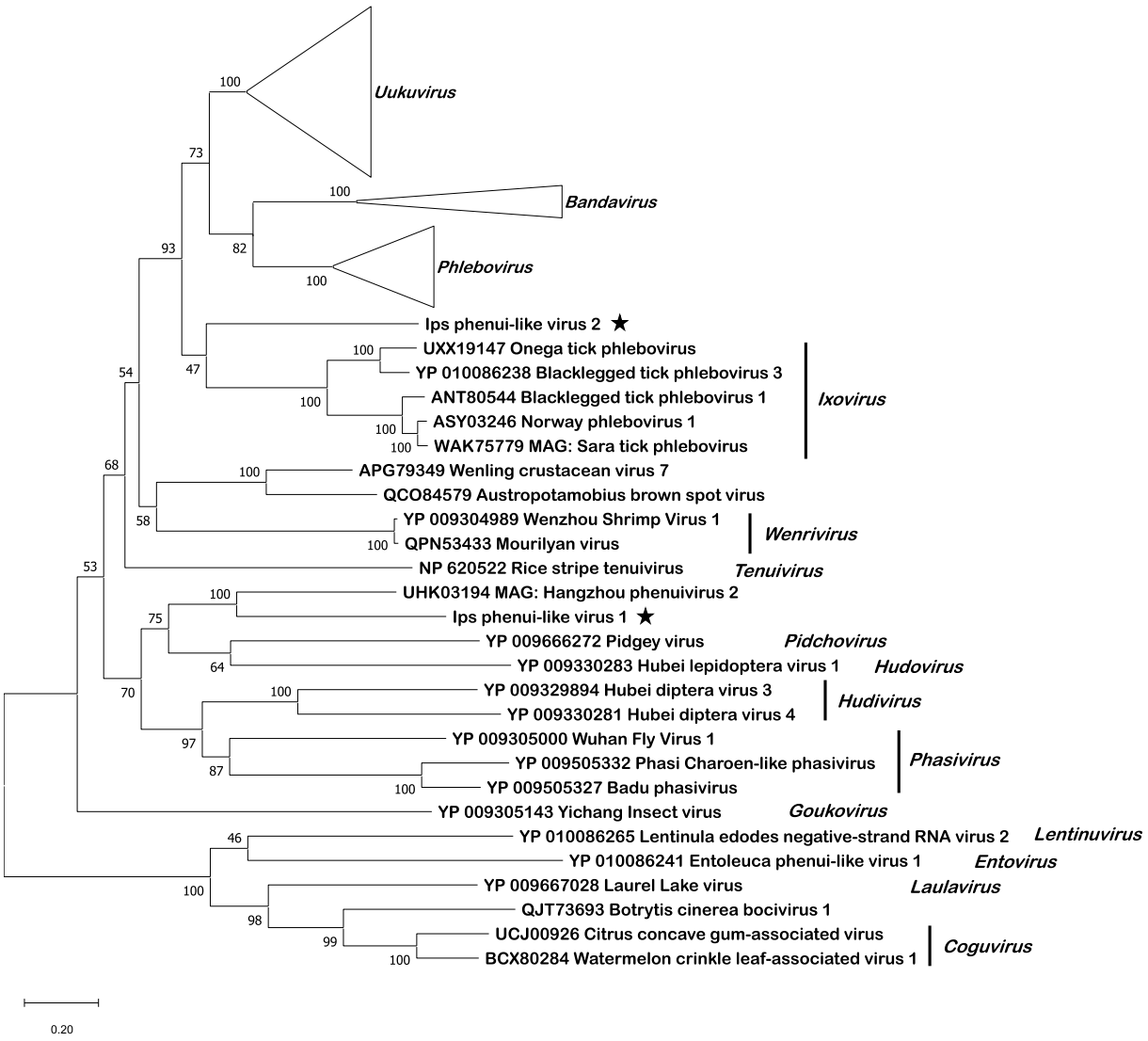


Fig. 8. A phylogenetic tree including of family *Phenuiviridae* and related unclassified viruses based on alignment of RdRP aa sequences as described in Fig. 2. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Stars denote the putative phenui-like viruses found in *Ips typographus*.

LTR-retrotransposons (PHA03247) and the large tegument protein UL36 (PHA03247). The 3' end of the sequence was apparently without ORFs, but a more thorough analysis of possible sequence polymorphisms revealed that the sequence downstream of the ORF1 included many positions with alternate numbers of tandem repeats that might break putative ORFs. However, testing of alternative numbers of tandem nucleotides did not result in generation of an ORF, and furthermore, Sanger sequencing suggested that the numbers of tandem repeat nucleotides downstream of nt 8121 corresponded to the reported contig sequence. That region of the sequence is assumed to encode a putative envelope protein in related insect viruses, such as Lampyris noctiluca errantivirus of the glow worm (a beetle species) (GenBank MH620823; Viljakainen et al., 2020).

Based on read mapping there were many SNPs in the errantivirus sequences (Table 1). Using the specific primers for the longest errantivirus sequence, PCR amplification yielded amplification products from both cDNA and non-reverse-transcribed nucleic acid samples, indicating that the virus was present in DNA form, which is in accordance with the integrated lifestyle of metaviruses.

3.5. Examining the possible presence of alternate hosts in the *I. typographus* holobiont

We utilized two strategies for examining the presence of reads originating from other organisms than *I. typographus* in our library: Blastx search against the nr database and read mapping against sequences of selected *I. typographus* associated taxa. Using a set of Trinity contigs showing no Blastn similarity with *I. typographus* with a length over 500 nt, a significant Blastx hit was found for 721 contigs. Almost half (48.8%) of the hits were assigned to orders Rhabditida and Strongylida within class Chromadorea indicating presence of nematode-originated reads in our library. Fungal derived contigs comprised 15.1% of the hits. Of fungal hits the protein sequences belonging to members of Kickxellomycotina, Mucoromycotina and Agarimycotina subdivisions were the most well represented followed by Chytridiomycetes, Pezizomycotina and Saccharomycotina hits. Bacterial sequences constituted 11.9% of all contigs mostly showing similarity to Gammaproteobacteria, Bacilli and Alphaproteobacteria proteins (3.9, 2.2 and 1.5%, respectively). Sequences derived from plants and insects (other than *I. typographus*) represented less than 4% of the Blastx hits. The remaining hits included contigs showing similarity with viral proteins (57 contigs) as well as metagenome assembled and/or third party data and suppressed

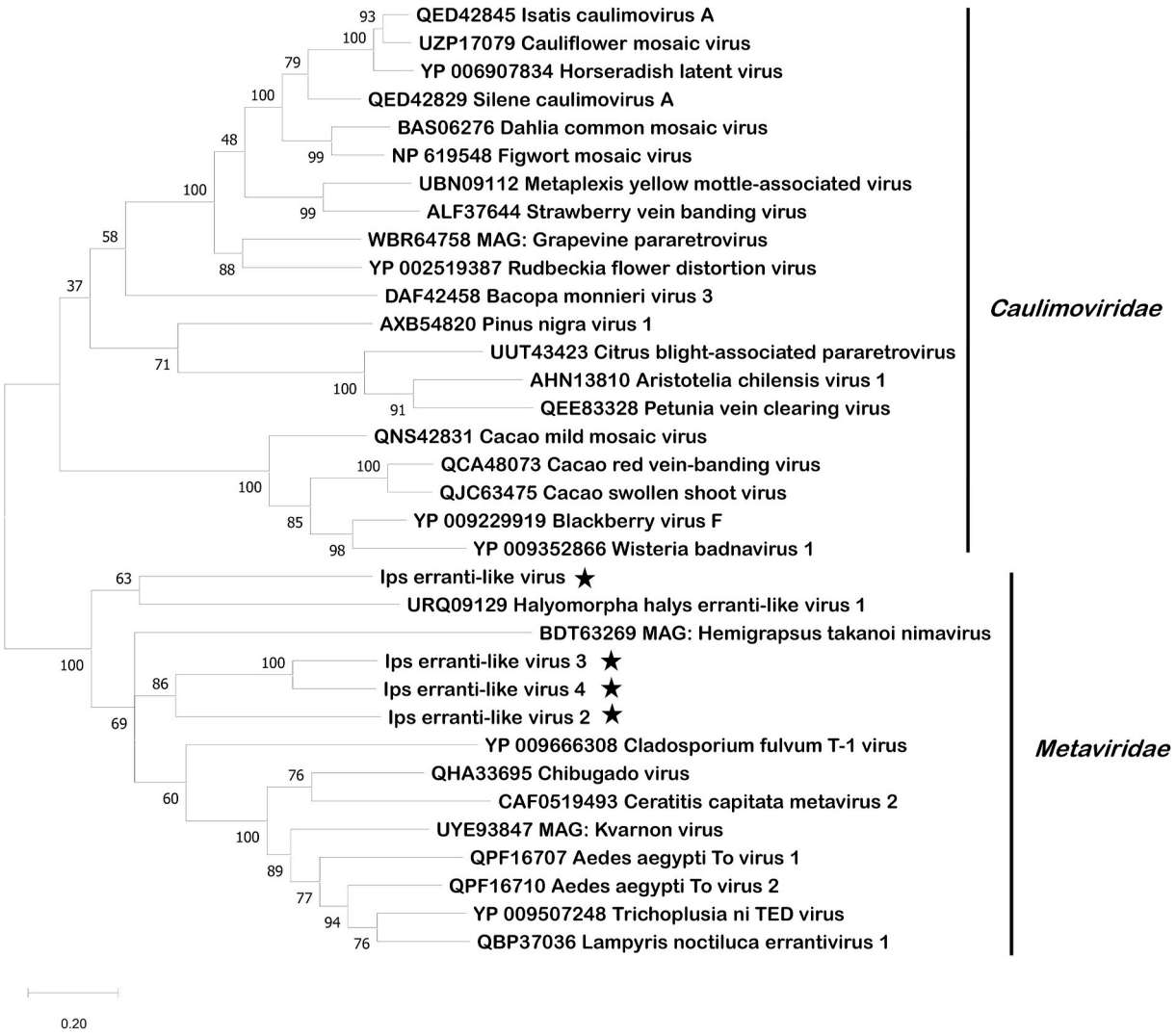


Fig. 9. A phylogenetic tree including selected viruses representing families *Metaviridae* and *Caulimoviridae* and related unclassified viruses based on alignment of replicase aa sequences as described in Fig. 2. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Stars denote the putative erranti-like viruses found in *Ips typographus*.

records (altogether 27 contigs).

Based on read mapping, the presence of nematodes of *Contortylenchus* spp. in the RNA sample seemed plausible. This was supported by continuous coverage of reference 28S rRNA sequences (GenBank DQ328731, KM245035, LC219519) by the reads, and the presence of a 2197 nt contig corresponding to *Contortylenchus* 28S rRNA (TRINITY_DN8232_c0_g1_i1; Supplementary Fig. S1). Similarly, one short contig (TRINITY_DN60118_c0_g1_i1; with the length of 302 nt) corresponded to *Cryptaphelenchus* spp. 28S rRNA (Fig. S1). In the case of most nematode or protozoan taxa known to be associated with *I. typographus* (*Bursaphelenchus* spp., *Parasitorhabditis* spp.; *Parasitolenchus* spp., *Mattesia* spp., *Gregarina* spp., see below), a number of raw reads were found to map against the reference sequences, but closer examination by Blast searches revealed that these were reads that matched only highly conserved rRNA locations and most probably originated from the beetle host.

4. Discussion

Insect DNA viruses with well-known etiologies have been successfully used as biocontrol agents to mitigate pest insect outbreaks, and on the other hand insects are known to vector many RNA viruses causing serious zoonoses in vertebrate animals. However, recent HTS studies have clearly demonstrated that a more inconspicuous part of the RNA virome of insects has remained mostly unknown. These newly discovered viruses may be insect-specific and infect their host persistently (Bolling et al., 2015), affecting host populations in yet unknown ways. Due to their ability to transmit serious human virus diseases, mosquito species have been studied more extensively than other insect groups by metatranscriptomics approaches thus far (Coatsworth et al., 2022). Shi et al. (2020) propose that insect-specific viruses in mosquitoes comprise of two different groups: a stable and species-specific “vertically transmitted core virome” stable across all developmental stages, and an “environment-derived core virome” which is less stable geographically and over time.

Nouri et al. (2018) present three different ways how persistent insect viruses could be used for biocontrol applications despite many of them are likely cryptic (asymptomatic) and well adapted to their insect hosts. Two approaches are based on controlling zoonotic viruses by virus-virus interference, either using a natural insect-specific virus to delimit the subsequent infection by a related zoonosis-causing virus (Goenaga et al., 2015) or by generating recombinant viruses to induce RNAi defenses against other viral pathogens vectored by the insect (Adelman et al., 2001). The third approach includes using engineered viruses, i.e., virus-induced gene silencing (VIGS) to target specific insect RNAs (see Joga et al., 2021 for a recent review on possibilities on wood-boring

coleopterans). In the case of *I. typographus*, the third approach could be developed towards a biocontrol application in case no viruses naturally pathogenic towards their host would be available.

The viruses detected in this study were not affiliated with RNA virus families containing viruses causing insect diseases (*Iflaviridae, Dicistroviridae, Nodaviridae, Alphatetraviridae, Rhabdoviridae, Reoviridae* and *Birnaviridae*; Ros et al., 2022). However, the tombus-like viruses were somewhat related to the unclassified chronic bee paralysis virus causing mortality in *Apis mellifera* (Olivier et al., 2008), and another virus group observed in *I. typographus* associated with diseases are the phenui-like viruses (Ros et al., 2022). However, considering the potential of using natural RNA viruses as biocontrol agents, phylogenetic analysis alone is not a good indicator of biocontrol potential, which needs to be examined by field and experimental studies.

To decipher whether novel viruses detected by HTS are hosted by the insect itself or another organism associated with the holobiont, it is essential to explore both the potential host range based on the presence of reads from other organisms, as well as phylogenetic association of the viruses. We found nematode-derived sequences to be relatively common in our dataset, and there was indication of *Contortylenchus* spp., and *Cryptaphelenchus* being associated with the beetles. Fungal reads were found only in relatively low quantity, and plant-derived sequences were even scarcer. Earlier studies have identified both nematodes and protozoa associated with *I. typographus* (Takov et al., 2006; Burjanadze et al., 2015; Wegensteiner et al., 2015b). However, the viromes of these organisms remain mostly unknown, although recent HTS-based analyses have expanded the view of viral diversity in soilborne nematodes (Vieira et al., 2022), and many plant viruses transmitted by nematode vectors are well documented. Protozoan viruses have been thus far investigated mostly in the context of human diseases (Barrow et al., 2020). The fungi associated with bark beetles include typically ophiostomatoid fungi (Linnakoski, 2011; Netherer et al., 2021), but also diverse non-specialized fungi such as mold-like fungi and yeasts (Muñoz-Adalia et al., 2017). Virus-infected wood decay fungi can remain infectious in the digestive tract of the large pine weevil (*Hylobius abietis*) (Drenkhan et al., 2013, 2016), and viruses are also known to occur in fungi carried by bark beetles (Andreou, 2021). Moreover, some insect and tick species can act as carriers of fungal viruses (Petrzik et al., 2016; Liu et al., 2016), but the topic has received little research and no similar examples have been published from bark beetles. Assessing the possible presence of plant viruses in beetle gut contents would greatly benefit from knowledge of the virome of the host plant, Norway spruce, which remains virtually unknown (Rumbou et al., 2021; Vainio et al., 2024).

Based on phylogenetic analysis, it is apparent that the evolutionary history of persistent insect-specific viruses is shared with many RNA viruses infecting distantly related hosts, such as fungi, oomycetes and plants (e.g., Shi et al., 2016; Ayllón and Vainio 2023), and for example in *Aedes* mosquitoes *Totiviridae* and *Partitiviridae* phylogenies suggest shared plant- and fungal-based lineages (Coatsworth et al., 2022). The viruses we detected in *I. typographus* represented several family level groups many of which have a high potential of inter-kingdom transmission at the evolutionary scale. However, in all cases the *I. typographus* viruses grouped among classified or recently found and yet unclassified arthropod viruses affiliated with these families, and none of the viruses were closely related to known myco- or plant viruses. Below, we will describe in detail current understanding on the potential host range of the viruses detected in *I. typographus* as deciphered based on phylogenetic analysis, starting with viral families and genera classically associated with insects (*Phenuiviridae,* Spiciviridae, "quenyaviruses" and *Errantivirus*), and continuing with families originally described in fungi (*Narnaviridae, Partitiviridae*) or plants (*Solemoviridae, Virgaviridae, Tombusviridae, Benyviridae*). It should be noted that there was no indication of bacteriophages in our dataset although bacteria seemed to be present in the samples. Moreover, viruses infecting bacteria are phylogenetically so distant from viruses of eukaryotes that there would be no doubt about their host range.

Phenuiviridae is a family accommodating mostly insect viruses, albeit the host range of the viruses classified in this family expands also to plants (genera *Coguvirus, Tenuivirus)* and fungi (genera *Entovirus, Lentinuivirus*)(see Kuhn et al., 2022 and the 9th ICTV Report available at https://ictv.global/report_9th/RNAneg/Bunyaviridae). In this study, the two phenui-like viruses found in *I. typographus* were related to but distinct from phlebo-like viruses found in arachnids (ticks or spiders). In phylogenetic analysis, Ips phenui-like virus 1 seemed to cluster separately from classified genera of *Phenuiviridae*, and Ips phenui-like virus 2 grouped as a separate sister clade to genus *Ixovirus* that accommodated tick-borne viruses, but seems to constitute a new genus. Arachnids (phoretic mites) are known to be associated with *I. typographus* (Moser et al., 1989), but as only gut contents and not whole insects were used for the RNA extraction, it seems more plausible that the phenui-like viruses are hosted by the beetle itself.

Classified members of virus family *Totiviridae* infect fungi or protozoa (King et al., 2011). However, besides the classified totiviruses, several family-level virus groups resembling members of *Totiviridae* have been identified from diverse hosts including insects (see e.g, Shi et al., 2016; Kondo et al., 2019; Ayllón and Vainio, 2023). A recent proposal for the reorganize the *Ghabrivirales* order (https://ictv.global/files/proposals/) accommodates these viruses in 15 new families and 12 new genera. Ips spici-like virus 1 is affiliated with the family designated as *Spiciviridae*, which contains numerous viruses from diverse arthropods (mosquitoes, thrips etc.), but also few plant-originating viruses (Tighe et al., 2022). The Palmetto virus possibly grouping within this clade is vertically transmitted in mosquitoes (Coatsworth et al., 2022).

Quenyaviruses comprise a new putative taxon recently identified by metagenomics only in arthropods, and possibly vertebrates and their associated parasites (Obbard et al., 2020). Thus far, very little is known about their host range, and information on transmission routes, tissue tropisms, or pathology is lacking.

Family *Metaviridae* includes retrotransposons and reverse-transcribing viruses with long terminal repeats. Metaviruses were originally discovered in the baker's yeast and drosophilid flies (Gypsy-like viruses in genus *Errantivirus*), and are considered to be ancestral to other families of reverse-transcribing viruses, such as *Caulimoviridae* and *Retroviridae* (Llorens et al., 2020). Amplification of errantivirus sequences from host DNA and the earlier reported presence of several retovirus-related transposons and jockey-like mobile element sequences in *I. typographus* genome (Powell et al., 2021) supports the notion that the errantivirus-like sequences found in this study are retroelements integrated in the beetle genome.

Members of family *Narnaviridae* have a monocistronic genome encoding only one protein, the RdRP. Numerous putative narna-like viruses with similar or more unconventional genome organizations have been found in fungi and oomycetes (see Ayllón and Vainio, 2023 and the references therein) as well as arthropods (Cook et al., 2013; Göertz et al., 2019), marine invertebrates (Shi et al., 2016), protozoa (Lye et al., 2016), and nematodes (Richaud et al., 2019). The narna-like viruses found in *I. typographus* were distantly related to each other and grouped in two different clades of narna-like viruses, both of which affiliated with proposed members of the putative genus "Betanarnavirus" (Dinan et al., 2020). Both of these unclassified clusters included a number of insect-derived viruses but also viruses infecting fungi and oomycetes. Harvey et al. (2019) identified a narna-like Sherlock virus resembling Ips narna-like virus 1 from native Australian fleas. Interestingly, they speculated that some viruses clustering with the Sherlock virus and reported from arthropods or marine invertebrates may actually infect trypanosomatids associated with these species. Notably, Harvey et al. (2019) detected numerous bacterial, fungal and protozoans associated with the analyzed fleas that were used the RNA-Seq analysis as whole.

Members of *Partitiviridae* are currently classified in four genera harbored by fungi, plants or protozoa (Nibert et al., 2014; Vainio et al., 2018). The partiti-like virus we detected in *I. typographus* is affiliated

with a new partitivirus genus tentatively named "Epsilonpartitivirus", which accommodates many insect-derived partitivirids obtained by metatransctiptomic approaches, as well as certain fungal and oomycete viruses (Shi et al., 2016; Nerva et al., 2017; Jiang et al., 2019). In some cases, the original host of the partitivirids detected from insects remais unclear: for example in thrips the host could be the insect itself but also a plant or fungus (Chiapello et al., 2021). However, Cross et al. (2020) demonstrated that there are also partiti-like viruses directly infecting insects: the galbut virus was shown to replicate in fly tissues and was transmitted vertically from both female and male flies. Similarly, verdadero virus of the mosquito *Aedes aegypti* depended on vertical transmission mode.

Classified members of *Tombusviridae, Solemoviridae* and *Virgaviridae* infect plants (Sõmera et al., 2021; Adams et al., 2017; ICTV taxonomy browser at https://ictv.global/taxonomy). However, recent metagenomics studies examining insect viromes have revealed numerous viruses that are related to but cluster outside the classified virus genera in these families. Paraskevopoulou et al. (2021) suggest that the *Tombusviridae* taxonomy should be revisited and have proposed accommodating the newly discovered insect-borne tombus-like viruses in three different family-level groups: the "aspovirus" clade (related to Ips tombus-like virus 1), the "surivirus" clade (resembling Ips tombus-like virus 2), and the "gopevirus" clade. Overall, the tombus-like viruses found in insects are genetically very diverse and appear to have either mono- (e.g., the Ips tombus-like viruses found in this study) or bisegmented genomes (e.g., Cook et al., 2013; Chiapello et al., 2021).

Plant sobemoviruses are transmitted mechanically or by beetle, aphid or mirid vectors (Sõmera et al., 2021). Many newly discovered sobemo-like viruses from insects (including the Ips virus we found in this study) are related to but distinct from classified plant sobemoviruses and also differ from their plant-infecting relatives in their genome organization (Shi et al., 2016; Chiapello et al., 2021). Also virga-like insect viruses cluster outside classified plant-infecting members of *Virgaviridae* (Adams et al., 2017). Moreover, the genome constitution seems to be different, and the Ips virga-like virus even contained a GP motif not found in classified members of *Virgaviridae*. Other virga-like viruses found in insects appear to have mono- or bisegmented genomes (Chiapello et al., 2021), with coding capacity for methyltransferase, helicase and RdRP domains. Plant virgaviruses encode these same replication proteins as well as capsid and movement proteins, and have 1–3 genome segments. Their vectors are plasmodiophorids (furovirus, pecluvirus, pomovirus), or nematodes (tobravirus).

Finally, classified members of *Benyviridae* infect plants and are vectored by root-infecting microbes of the Plasmodiphorales family, currently classified as Cercozoa (Gilmer et al., 2017). They have segmented genomes and cluster separately in phylogenetic analysis from newly discovered insect-derived beny-like viruses that seem to possess only one genome segment encoding an RdRP and a coat protein (Etebari et al., 2022). As the insect beny-like viruses detected thus far are reported from metagenomics studies, their biology remains unknown.

This study showed that there is a previously unknown community of RNA viruses associated with *I. typographus*. Taking into account that all the beetles collected for the study originated from a single forest site, the viral diversity we detected was considerable. However, we expect that *I. typographus* harbors viruses of many more families and envisage future studies examining how viral communities vary among geographical regions, seasons and though insect developmental stages. That type of knowledge would be a prerequisite for determining whether the viruses have adverse effects on their host and whether they have potential for future biocontrol applications. The search for viruses with potential to regulate their hosts' populations should start by looking for differences in the bark beetle viromes at forest sites with periodic bark beetle damages without leading to major outbreaks, and those of outbreak populations. More research is also needed on viral communities of fungi associated with bark beetles, which would help deciphering which of the viruses detected in bark beetles are *bona fide* insect viruses and might have a potential to affect their host directly. If viruses associated with lower beetle population levels are discovered, there are still many research questions to be solved, for example concerning practical application of viral preparations on host insects that have extensive distribution ranges and whose population levels are largely determined by the availability of suitable breeding material (stressed host trees).

CRediT authorship contribution statement

Suvi Sutela: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Juha Siitonen:** Investigation, Resources, Validation, Writing – original draft, Writing – review & editing. **Tiina Ylioja:** Investigation, Resources, Validation, Writing – original draft, Writing – review & editing. **Eeva J. Vainio:** Conceptualization, Formal analysis, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2024.106706.

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