Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Comparing virome composition between honey bees (Apis mellifera) and small carpenter bees (Ceratina calcarata) in response to various agricultural landscapes

Zixiao Zhao
Central State University
Rameshwor Pudasaini
Central State University
M. Catherine Farrell
Central State University
Jayla Marvin
Central State University
Sandra M. Rehan
York University
Hongmei Li-Byarlay
Hli-byarlay@centralstate.edu

Central State University

Article

Keywords: Honey bees, agricultural landscape, transcriptomics, bee virus, iflavirus, entomopoxvirus

Posted Date: September 7th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10753067/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Insect pollinators face growing challenges from viral pathogens that may contribute to population declines, yet comparative data on virome composition in managed versus wild bees remain limited. Using RNA-Seq-based metatranscriptomic analysis, we compared the virome of *Apis mellifera* L. and *Ceratina calcarata* Robertson collected from agricultural and non-agricultural landscapes. We identified multiple known bee pathogens including Black Queen Cell Virus and Deformed Wing Virus in *A. mellifera*, and discovered three putative novel insect viruses-two iflaviruses and one entomopoxvirus-associated with *C. calcarata*. Notably, one putative novel iflavirus was identified in *A. mellifera*. Our findings reveal species- and landscape-specific viral prevalence, highlight viral diversity beyond canonical bee pathogens, and provide genomic resources for future functional studies. These data offer new insights into virus-host dynamics and underscore the utility of virome profiling in understanding pollinator health at the molecular level.

INTRODUCTION

Animal pollinators play critical roles in maintaining biodiversity and ecosystem stability worldwide (Katumo et al., 2022; Ollerton, 2017). Approximately 87.5% of floral plant species depend on animal-mediated pollination for successful reproduction (Ollerton et al., 2011), and around 75% of crop species rely on pollinators to enhance yield and quality, contributing roughly 30–35% of global agricultural output (Khalifa et al., 2021; Klein et al., 2007). Among these pollinators, bees (Hymenoptera: Apidae) are the important and efficient, with honey bees (*Apis* spp.) alone accounting for an estimated 80% of total pollination services and generating approximately $700 million in annual economic value in the United States (Breeze et al., 2011).

Yet reliance on *Apis* species masks the critical, often underappreciated contributions of wild bee communities. Wild bees, comprising over 17,000 species globally, can complement or even surpass honey bees in pollination efficiency, especially under conditions where managed honey bee populations are compromised (Brittain et al., 2013; Winfree et al., 2007). These wild species exhibit diverse life histories and social structures, from solitary to eusocial, which can influence their exposure and vulnerability to emerging stressors, including viral pathogens (Dolezal et al., 2016; Tehel et al., 2016). Previous reports showed that bee viruses were detected in families of Andrenidae, Apidae, Halictidae, and Megachilidae but the viral load was low in wild bees compared to honey bees (Dolezal et al., 2016; Deutsch et al., 2023; Tehel et al., 2016). Other research indicated that bumblebees are also infected by honey bee viruses (Toplak et al., 2020).

Over the past two decades, both managed and wild bee populations have experienced significant declines, with consequences not only for agricultural production but for broader ecosystem integrity and food security (Drossart and Gérard, 2020; Goulson et al., 2015; Potts et al., 2010; Roulston and Goodell, 2010). Multiple stressors, including habitat loss, pesticides exposure, climate change, and the spread of pathogens, act synergistically to threaten bee heath (Brown et al., 2016; Siviter et al., 2023; Guo et al., 2025). Among these, viruses have emerged as a particularly insidious threat due to their ability to spread within and across species via shared floral resources and environmental contamination (Singh et al., 2010; Zhang et al., 2012; Ullah et al., 2021).

The majority of known pathogenic bee viruses are positive-sense single-stranded RNA viruses, with most belonging to the families *Iflaviridae* (e.g., Deformed Wing Virus (DWV), Slow bee paralysis virus (SBPV), Sacbrood Virus (SBV), and *Dicistroviridae* (e.g., Black queen cell virus (BQCV), Kashmir bee virus (KBV), Acute bee paralysis virus (ABPV), and Israeli acute paralysis virus (IAPV)) (Walker et al., 2022; Li-Byarlay et al., 2020). Additional important viruses include Chronic bee paralysis virus (CBPV), which remains unclassified (Seitz et al., 2019), and Lake Sinai viruses (LSVs), of the order Nodamuvirales (Walker et al., 2022, Chen et al., 2023). These viruses can cause a range of symptoms from deformities to neurological impairment and colony collapse, and are often linked with other stressors such as parasite mite *Varroa destructor* infestations or intensive land use (Cox-Foster et al., 2007).

While most studies have focused on honey bee viromes, recent evidence indicates that wild bee species, including solitary and subsocial species, are frequently infected with the same viruses (Singh et al., 2010; Dolezal et al., 2016; Tehel et al., 2016). DWV, for instance, has been identified in both eusocial and solitary bees and wasps, suggesting that viral host range may be broader than previously thought and influenced by environmental context (Singh et al., 2010; Tehel et al., 2016; Zhang et al., 2012). Dolezal et al. (2016) reported DWV and SBV were widely detected in wild bees collected in Iowa, while IAPV, LSV and BQCV were also detected in wild be species. Furthermore, DWV, BQCV and SBV can be deposited into pollen and remain infective, which can contribute to inter-taxa transmission among bees, and wasps (Singh et al., 2010). These studies suggested viruses can be a potential factor responsible for colony declines in wild bee species.

Agricultural intensification alters landscape structure, floral diversity, pesticides exposure, and microbial exposure, and may therefore influence host-pathogen dynamics in pollinator communities (Susi and Laine, 2021). Agricultural intensification and monoculture often reduce floral diversity and resource availability for pollinators, concentrating them on limited floral resources (Doublet et al. 2022; Proesmans et al., 2021). Such aggregation can facilitate horizontal transmission of viruses among individuals and across species (Dalmon et al., 2021). Managed honey bees, widely deployed in agricultural systems, may further amplify viral prevalence and act as reservoirs for spillovers into wild bees (Piot et al., 2022). On the other hand, organic management systems, which limit pesticide use and promote floral diversity, may therefore support different viral communities in pollinators compared to conventional systems. In contrast, roadside habitats often unmanaged and exposed to vehicle emissions and fragmented floral resources represent a distinct environment that can serve as both refugees and transmission zones for bee viruses. Diverse landscapes may dilute transmission by supporting greater plant diversity, reducing pollinator overlaps, and limiting viral persistence in the environment (Doublet et al. 2022). Understanding how these contrasting habitats shape the bee viromes can reveal how human land-use practices influence pollinator disease ecology.

The small carpenter bee, *Ceratina calcarata* Robertson (Hymenoptera: Apidae), is a wild bee species native to the eastern part of North America. It is considered as an indicator species of healthy ecosystems and is an important pollinator for several native and agricultural plants (Lawson et al., 2021). Owing to its subsociality nature, *C. calcarata* is widely used as a model species in studies of pollinator ecology, behavior, evolution, genomics and nutrition (Lawson et al., 2017; Rehan et al., 2016; Rehan and Richards, 2010; Shell and Rehan, 2017). However, little is known about the virology of this species. The impact of viruses on *C. calcarata* populations, and whether the viral prevalence is influenced by agricultural activities and interspecies interactions with *A. mellifera*, remain unclear.

Here we presented metagenomic studies on viruses associated with *A. mellifera* and *C. calcarata* in response to different agricultural intensifications. This study hypothesizes that differences in land management practice and habitat context influence the diversity and composition of bee-associated viruses, due to differences in floral diversity, pesticide exposure, and host interactions; and shared foraging habitats promote viral overlap between managed and wild bees. Specifically, we predict that bees from organic farms will exhibit greater viral diversity but lower viral loads due to more diverse floral resources and reduced pesticide exposure, while bees from conventional farms and roadside habitats will harbor higher loads of common viruses associated with environmental stress and limited floral resources.

MATERIAL AND METHOD

Sample collection

Both honey bee (*A. mellifera*) and small carpenter bee (*C. calcarata*) samples were collected from habitats representing three different types of land use: conventional farms, organic farms and roadside habitats. To minimize age-related variation, forager bees of both *A. mellifera* and *C. calcarata* were collected by sweep nets from May to July 2018 in Strafford County, New Hampshire. We collected both species within three conventional farms and three organic farms. The roadside samples were collected in the urbanized area within the University of New Hampshire campus. The details about locations and GPS coordinates are described in previous publications (Tucker and Rehan, 2017, 2018).

At least 40 individuals per bee species were collected at each sampling site, and collected bees were flash-frozen in liquid nitrogen and stored at -80°C. In total, at least 120 *A. mellifera* and 120 *C. calcarata* individuals were collected across all sampling sites. From these, RNA was extracted and used for RNA-sequencing from 33 pooled samples that include 17 *A. mellifera* and 16 C. *calcarata*. Details on the number of individual bees used for RNA-sequencing from each collection site and additional information are provided in **Supplementary Table S1**.

RNA extraction, sequencing and data cleaning

The viral RNA was extracted from *A. mellifera* and *C. calcarata* individually by Quick-RNA viral kit (ZYMO RESEARCH, Irvine, CA). DNase I (ZYMO RESEARCH) in-column digestions were applied to remove genomic and environmental DNA. For mRNA library generation, 200 ng of total RNA was treated with NEBNext Poly mRNA Magnetic Isolation Module E7490L (New England Biolabs, USA) following the manufacturer protocol. Subsequently, the isolated mRNA was fragmented for 10 minutes. cDNA was synthesized and amplified for 12 PCR cycles using NEBNext Ultra II Directional (stranded) RNA Library Prep Kit E7760L for Illumina (New England Biolabs, USA) with NEBNext Multiplex oligos indexes kit E6440S following the manufacturer directions. Distributions of the template length and adapter-

dimer contamination were assessed using an Agilent 2100 Bioanalyzer and High Sensitivity DNA kit (Agilent Technologies, Inc). The concentration of cDNA libraries was determined using Invitrogen Qubit dsDNA HS reagents and read on a Qubit Fluorometer (Thermo Fisher), and the cDNA libraries were paired-end 150bp format sequenced on a Novaseq SP system (Illumina, San Diego, CA). In total, 17 honey bee samples and 16 *C. calcarata* samples were sequenced (**Supplementary File S1**). Raw reads were subject to adapter and quality trimming by Trimmomatic (v0.040) (Bolger et al., 2014). Sample metadata from collection was used to assign each sample to either *A. mellifera* or *C. calcarata*. To remove endogenous transcripts from bee hosts, the reads were respectively aligned to honey bee and small carpenter bee (Rehan et al., 2016) genomes (including mitochondrial sequences) using Bowtie2 (v2.5.4) (Langmead and Salzberg, 2012). Read pairs that were concordantly aligned to bee genomes were considered as endogenous transcripts and removed from further analysis.

Virome assembly and viral identification

To reconstruct the virome of *A. mellifera* or *C. calcarata*, we first merged all the pre-cleaned reads from the same organism to increase the sequence coverage. *De novo* assemblies were performed using Trinity (v2.13.2 release series) (Grabherr et al., 2011) and metaSPAdes (SPAdes v4.2.0) (Nurk et al., 2017) were used with default parameters. The results were comparable; however, the total running time for Trinity was lower. Therefore, we chose Trinity for the subsequent analyses.

Despite the pre-cleaning process, the assembled virome still contains host sequences. To identify virus sequences, we used BLASTN (v2.15.0) to search for assembled contigs to default nr/nt database with E-value cutoff of 1e-7. Contigs with first three top hits to viral sequence from database was retained as viral contigs. For further virus identification, we examined the blast results of contigs > 500 nt. A contig was assigned as a known virus if it aligned stringently (E-value = 0) to existing viruses. If the alignment is less stringent (0 < E-value < 1e-7), it was assigned as a putative novel virus. The “full-length” or “partial” labels were based on the completeness and intactness of viral protein of each viral contig. For potential novel viral contigs, ORF finder and BLASTP (v2.15.0) were used to translate and predict viral protein. Maximum-likelihood phylogenies were generated in MEGA 11 (v11) using the best-fit amino acid substitution model selected under the Akaike Information Criterion (AIC) (Tamura et al., 2021). Bootstrap support values were calculated from 500 replicates. Gaps and missing data were treated by pairwise deletion.

Abundance of viruses and differential analysis between landscapes

To quantify the abundance of each contigs, the clean reads from each sample were mapped to the virome assembly of respective bee species using Bowtie 2 (2.5.4) (Langmead and Salzberg, 2012) in sensitive-local mode, allowing multiple high-scoring alignments per read. Multiple alignment was allowed to obtain maximum sensitivity. Read counts were obtained by FeatureCounts (v2.0.7) (Liao et al., 2014) and normalized to count per million reads (CPM) with R edgeR package (v4.0) (Robinson et al., 2010). Prior to statistical analyses, each contig, including endogenous and viral, was filtered based on its expression level. Contigs were retained if they had a minimum expression of ≥ 1 CPM in at least 11 samples (Chen et al., 2016). Differential abundance analysis between landscape types was conducted in edgeR using a negative binomial model, and contigs with an FDR < 0.05 were considered significantly different. Only contigs previously identified as viral were included in downstream interpretation.

RESULTS

Reconstruct virome from viral RNA-Seq reads

The RNA-Seq of extracted viral RNA generated approximately 17 to 36 million raw read pairs for each *A. mellifera* and *C. calcarata* sample. After quality trimming and partially removing reads from respective host genomes (Amel HAv3.1 PRJNA471592 and Ccalc.v3 PRJNA791561), 5 to 25 million pairs were kept for each sample (**Supplementary file S1**). Trinity *de novo* assembled 366,289 contigs for *A. mellifera* and 203,317 contigs for *C. calcarata* virome, which consists of 188,433 and 91,314 non-redundant clusters. The assembled contigs were subject to BLASTN searches against non-redundant databases (nr), which claimed 541 clusters and 1187 contigs from *A. mellifera* assembly potentially belong to viruses. In contrast, 62 clusters and 84 contigs from the *C. calcarata* assembly were potentially from viruses. More than 50% of assembled contigs were from either host genes or plants they have visited.

Viruses infecting bees and other insects detected in A. mellifera and C. calcarata

BLASTN search identified four known bee pathogenic viruses from *A. mellifera* virome: BQCV, DWV, SBV, and LSV3 with fully assembled genome sequences. Two other members of the LSV group: LSV1 and LSV2 were detected, but their sequences were only partially assembled. Positive and negative strands were detected in all of the above viruses except LSV2, from which only positive strand was detected. We also detected *Apis mellifera* filamentous virus (AmFV) (Hartmann et al., 2015) and Hubei partiti-like virus 34 in *A. mellifera*. The AmFV were partially assembled and the Hubei partiti-like virus 34 demonstrated a fully assembled genome (Table 1). In *C. calcarata*, BQCV was the only detected bee pathogenic virus and appeared as partial-assembled as negative-strand contig (Table 2).

Table 1
List of identified insect and plant viruses in *A. mellifera* virome assemblies, assembled from RNA-Seq data

Virus name	Abbreviation	Type	Family	Genus	Host	Assemble status	Strand
Black queen cell virus	BQCV	(+)ssRNA	Dicistroviridae	*Triatovirus*	Bees	Full	+/-
Deformed wing virus	DWV	(+)ssRNA	Iflaviridae	*Iflavirus*	Bees	Full	+/-
Sacbrood virus	SBV	(+)ssRNA	Iflaviridae	*Iflavirus*	Bees	Full	+/-
Lake Sinai virus 3	LSV3	(+)ssRNA	Sinhaliviridae	*Sinaivirus*	Bees	Full	+/-
Lake Sinai virus 1	LSV1	(+)ssRNA	Sinhaliviridae	*Sinaivirus*	Bees	Partial	+/-
Lake Sinai virus 2	LSV2	(+)ssRNA	Sinhaliviridae	*Sinaivirus*	Bees	Partial	+
Apis mellifera filamentous virus	AmFV	dsDNA	*unclassified*	*unclassified*	Bees	Partial	+/-
Hubei partiti-like virus 34	-	RNA	*unclassified*	*unclassified*	Bees	Full	+
Blackberry chlorotic ringspot virus	BCRV	(+)ssRNA	Bromoviridae	*Ilarvirus*	Plants	RNA1: Full	+
						RNA2: Partial	-
						RNA3: Partial	+
Tobacco streak virus	TSV	(+)ssRNA	Bromoviridae	*Ilarvirus*	Plants	RNA1: Partial	+/-
						RNA2: Partial	+/-
						RNA3: Partial	+
Peanut stunt virus	PSV	(+)ssRNA	Bromoviridae	*Cucumovirus*	Plants	RNA1: Partial	+
						RNA2: Partial	+
						RNA3: Partial	-
Raphanus sativus cryptic virus 1	RasCV1	dsRNA	Partitiviridae	*unclassified*	Plants	RNA1: Full	-
						RNA2: Missing	
White clover cryptic virus 1	WCCV-1	dsRNA	Partitiviridae	*Alphapartitivirus*	Plants	RNA1: Full	-
						RNA2: Full	-
Virus name		**Abbreviation**	**Type**	**Family**	**Genus**	**Host**	**Assemble status** **Strand**

Virus name	Abbreviation	Type	Family	Genus	Host	Assemble status	Strand
White clover cryptic virus 2	WCCV-2	dsRNA	Partitiviridae	*Alphapartitivirus*	Plants	RNA1: Full	-
						RNA2: Full	-
Red clover nepovirus A	RCNVA	(+)ssRNA	Secoviridae	*Nepovirus*	Plants	RNA1: Partial	+
						RNA2: Partial	+/-
Tobacco ringspot virus	TRSV	(+)ssRNA	Secoviridae	*Nepovirus*	Plants	RNA1: Partial	+/-
						RNA2: Partial	-
Tomato ringspot virus	ToRSV	(+)ssRNA	Secoviridae	*Nepovirus*	Plants	RNA1: Partial	+/-
						RNA2: Partial	-
White clover mosaic virus	WClMV	(+)ssRNA	Alphaflexiviridae	*Potexvirus*	Plants	Full	+/-
Clover yellow mosaic virus	ClYMV	(+)ssRNA	Alphaflexiviridae	*Potexvirus*	Plants	Partial	+/-
Red clover vein mosaic virus	RCVMV	(+)ssRNA	Betaflexiviridae	*Carlavirus*	Plants	Partial	+/-
Pinus flexilis virus 1	PiFleV1	(-)ssRNA	Rhabdoviridae	*unclassified*	Plants	Partial	+/-

Table 2
List of identified insect and plant viruses in *C. calcarata* virome assemblies, assembled from RNA-Seq data

Virus name	Abbreviation	Type	Family	Genus	Host	Assembly status	Strand
Black queen cell virus	BQCV	(+)ssRNA	Dicistroviridae	*Triatovirus*	Bees	Partial	-
Tomato ringspot virus	ToRSV	(+)ssRNA	Secoviridae	*Nepovirus*	Plants	RNA1: Partial	+/-
						RNA2: Partial	+/-
White clover cryptic virus 1	WCCV-1	dsRNA	Partitiviridae	*Alphapartitivirus*	Plants	RNA1: Full	-
						RNA2: Missing	
White clover cryptic virus 2	WCCV-2	dsRNA	Partitiviridae	*Alphapartitivirus*	Plants	RNA1: Full	+
						RNA2: Full	-
White clover mosaic virus	WClMV	(+)ssRNA	Alphaflexiviridae	*Potexvirus*	Plants	Partial	+/-

Plant viruses discovered in A. mellifera and C. calcarata virome assemblies

A range of plant viruses were detected in virome assemblies of both bee species. In *A. mellifera*, white clover cryptic virus 1 (WCCV-1) and white clover cryptic virus 2 (WCCV-2) from genus *Alphapartitivirus* (family: Partitiviridae, +ssRNA), as well as white clover mosaic

virus (WCMV) from genus *Potexvirus* (family: Alphaflexiviridae, +ssRNA) were fully assembled. Blackberry chlorotic ringspot virus (BCRV) from genus *Ilarvirus* (family: Bromoviridae, +ssRNA) and *Raphanus sativus* cryptic virus 1 (RsPV1) from Partitiviridae (+ ssRNA) have one genomic segment fully assembled, while other segments were either partially assembled or completely missing. Partially assembled plant viruses include 7 + ssRNA viruses: peanut stunt virus (PSV) and tobacco ringspot virus (TRSV) from Bromoviridae, clover yellow mosaic virus (ClYMV) from Alphaflexiviridae, red clover nepovirus A (RCNVA), tobacco streak virus (TSV), and tomato ringspot virus (ToRSV) from genus *Nepovirus* (family: Secoviridae), red clover vein mosaic virus (RCVMV) from Betaflexiviridae, and one -ssRNA virus: *Pinus flexilis* virus 1 (PiFleV1) from Rhabdoviridae (Table 1). However, the plant-associated virome of *C. calcarata* was considerably less diverse compared to *A. mellifera*. WCCV-2 was the only fully assembled plant virus, while WCCV-1, BCRV, ToRSV, and WClMV were partially assembled in *C. calcarata* (Table 2).

Putative novel viruses in A. mellifera and C. calcarata virome assemblies

The novel viruses detected in *A. mellifera* and *C. calcarata* virome assemblies assembled from RNA-Seq data are presented in Table 3. The genus *Iflavirus* (family: Iflaviridae) consists of +ssRNA viruses infecting arthropods, including three bee pathogenic viruses SBV, DWV, and SBPV. Two putative novel viruses under genus *Iflavirus*, referred to as unknown iflavirus 1 and unknown iflavirus 2, were identified in *C. calcarata* virome assembly as positive strands. The phylogenetic analysis using translated polyprotein amino acid sequence suggests both viruses are in the same group as SBV. The unknown iflavirus-1 has the highest identity with Darwin bee virus-2, an unclassified picornavirus infecting honey bees (Roberts et al., 2018) (Fig. 1A). This virus was also detected in *A. mellifera* virome assembly as a negative strand, indicating active replication in honey bees.

Table 3
List of putative novel viruses detected in *A. mellifera* and *C. calcarata* virome assemblies assembled from RNA-Seq data

Virus	Potential host	Length (bp)	Viral protein ORF status	Detected in
Merkel cell polyomavirus	Vertebrate	3330	Partial	*A. mellifera*
		10809	Full	*C. calcarata*
Unknown iflavirus 1	Arthropods	1574	Partial	*A. mellifera*
		616	Partial	*C. calcarata*
Unknown iflavirus 2	Arthropods	7343	Full	*C. calcarata*
Unknown nepovirus 1	Plants	6501	Full	*A. mellifera*
Unknow partitivirus 1	Plants	1895	Full	*A. mellifera*
Unknown amalgavirus 1	Plants	3425	Full	*A. mellifera*
Unknown entomopoxvirus 1	Arthropods	2543	Partial	*C. calcarata*

In *C. calcarata*, we detected a putative novel poxvirus (a double-strand DNA virus). Its DNA-dependent RNA polymerase RPO132 is partially assembled. Phylogenetic analysis showed this virus is closely related to the Yalta virus, an uncategorized poxvirus from *Drosophila melanogaster* and *Diachasmimorpha* entomopoxvirus (DIEPV), from parasitoid wasp *Diachasmimorpha longicaudata*. The three viruses form a sister cluster to other entomopoxviruses (EPVs) (genus: *Alphaentomopoxvirus*, *Betaentomopoxvirus*, and *Deltaentomopoxvirus*), while all the vertebrate poxviruses (genus: *Avipoxvirus*, *Capripoxvirus*, and *Parapoxvirus*) are clustered into a separate group. The result suggests this putative novel virus belongs to the subfamily Entomopoxvirinae (EPV) that primarily infect insects (Fig. 1E).

Three additional putative novel plant-associated viruses were detected in the *A. mellifera* virome, including one from the genus *Nepovirus* (unknown nepovirus 1, family: Secoviridae), one from family Partitiviridae (unknown parititivirus 1, family: Partitiviridae), and one of genus *Amalgavirus* (unknown Amalgavirus 1, family: Amalgaviridae). Nepovirus has two positive-strand genomic RNAs (Rowhani et al., 2017). For unknown nepovirus 1, the open reading frame (ORF) of polyprotein RNA2 was fully assembled. However, the correspondent RNA1 encoding viral coat protein was not detected. The phylogeny of the polyprotein sequence shows that it is most closely related to Blueberry leaf mottle virus and Grapevine Bulgarian latent virus, forming a separate cluster (Fig. 1B). Similarly, only RNA1 encoding RNA-dependent RNA polymerase (RdRp) was fully assembled for another potential plant virus: unknown parititivirus 1. The phylogenetic analysis of RdRp showed that it is clustered with several uncategorized partitivirus viruses associated with plant:

Polygonatum kingianum cryptic virus 1, Panax cryptic virus 3, and *Dichroa partitivirus* 1. This cluster is a sister group to plant-infecting genus *Alphapartitivirus* (Fig. 1D). The genome of unknown amalgavirus 1 was fully assembled with two complete ORF: the ORF1 + 2 fusion protein responsible for genome replication, and a coat protein. The phylogeny of fusion proteins showed that it is the most closed to Vicia cryptic virus M (VCV-M) and Medicago sativa amalgavirus 1 (MsAV1) and have a clear separation with *Zygosaccharomyces bailii* virus Z (ZbV-Z), a yeast-infecting virus belongs to the genus *Zybavirus* (Fig. 1C).

In addition, other viruses, including phages and a human virus: Merkel cell polyomavirus (Family: Polyomaviridae) were identified in both virome assemblies. The viral genome consists of circular double-stranded RNA (dsRNA), which resulted in the assembly of two identical full-length viral sequences into one contig in the *C. calcarata* virome. In *A. mellifera*, only partial sequence was assembled. How the Merkel cell polyomavirus was introduced into the virome and its effects in both species, are still unknown.

Abundance of viruses in response to different agricultural landscapes

The distributions of the above detected viruses were site-specific. In *A. mellifera*, among all identified insect viruses, five known bee viruses: BQCV, DWV, LSVs, SBV, and AmFV were detected at all of the sampling sites regardless of agricultural practices, with AmFV contigs detected < 1 CPM in conventional farm C1. In contrast, the putative novel unknown iflavirus 1 was only detected in samples from organic farm O2 (Fig. 2A). The five-known bee viruses detected in this study did not show any significant differences in levels across the different agricultural landscapes. However, a separate 2834-bp LSV3 segment, containing ORFs of two viral proteins, was significantly increased in bees collected from organic farms compared to bees collected from roadside habitat (FDR: 0.04). A near full-length sequence (~ 3.4kb) of Hubei partiti-like virus was also identified at all sites except conventional farm C1 (Fig. 2A). The sequence was significantly higher in bee samples collected from organic farms compared to bee samples collected from both conventional farms and roadside habitats (FDR: 0.006 to conventional, 0.003 to roadside).

The presence and levels of plant viruses were highly dependent on the sampling locations. For instance, TSV and PiFleV1 were detected only at one separate roadside habitat each, while RCNVA and TRSV were found only at one conventional and one organic farm, respectively. RCVMV was identified across three conventional farms, but at relatively low levels. WCIMV, WCCV-2, and ToRSV were detected at all sampling sites (Fig. 2B). WCCV-1 was the only significant plant virus detected by edgeR. The levels of both genome segments decreased significantly in organic farms, compared to both conventional farms (FDR, RNA1: 0.011, RNA2: 0.006) and roadside habitats (FDR, RNA1: 0.006, RNA2: 0.003). The viral levels of BRCV at roadside habitats were either very low or undetected, but the overall level is too low to perform statistical analysis in edgeR.

C. calcarata hosted relatively fewer viruses compared to *A. mellifera*. The presence of insect viruses was also highly habitat-specific. BQCV and unknown entomopoxvirus 1 were only found at one conventional farm, whereas two putative novel iflavirus were detected in roadside habitats and conventional farm C1. None of the viruses were detected from bee samples collected from organic farms, conventional farm C3, and roadside R2 site (Fig. 2C). Regarding the plant viruses, WCCV-1 and WCCV-2 had a similar pattern, being detected only at conventional farms, while WCIMV and ToRSV were detected in few bee samples collected from roadside habitats as well (Fig. 2D). However, their levels were not statistically significant. Similarly, none of the plant viruses were detected in bee samples collected from organic farms.

DISCUSSION

The present study was designed to examine how differences in land management and habitat type influence the diversity and composition of bee-associated viruses. Specifically, we investigated whether viral communities differ among organic farms, conventional farms, and roadside habitats, and whether viral overlaps between managed and wild bee species in shared environments indicate potential interspecies viral exchange. This study surveyed the abundance of viruses associated with *A. mellifera* and *C. calcarata*. Findings show that the virome compositions were habitat-specific. However, the *A. mellifera* carried a wider range of both insect and plant viruses than *C. calcarata*. The detection of multiple plant-associated virus sequences in *A. mellifera* suggests a higher level of exposure to plant-associated viruses compared to *C. calcarata*. While this may reflect differences in foraging behavior in these two-bee species, our study does not provide evidence about their ability to transmit these viruses. Furthermore, this study relied on RNA sequencing and the detection of both positive and negative strands, which helps distinguish actively replicating viruses from integrated viral elements. However, presence of endogenous viral sequences cannot be entirely excluded.

The levels of BQCV, DWV and SBV were high in the *A. mellifera* virome across all sampling sites, suggesting a high prevalence of these viruses in *A. mellifera*. In contrast, none of the acute bee paralysis virus complexes (ABPV, KBV and IAPV) were detected. This result is consistent with other studies in the Northeast of the US (Drummond et al., 2021; Singh et al., 2010). LSV is a highly variable, multi-strain bee virus that does not have any significant symptoms but is likely to weaken colonies. The LSV contigs assembled in this study are most closely related to LSV1, LSV2 and LSV3. The LSV3 was detected at all sampling sites and the only LSV with full-length genome sequence assembled. The LSV3 was originally identified in Slovenia (Šimenc et al., 2020) and has been detected in the Western US (Daughenbaugh et al., 2015). The assembled sequence clustered with published LSV3 genomes, supporting the presence of LSV3 (or a very closely related variant) in the New England area.

Two known viruses associated with *A. mellifera* were also detected: AmFV and Hubei partiti-like virus 34. The AmFV is considered a weak pathogen in *A. mellifera*, and no direct association between this virus and colony collapse disorder (CCD) in honey bees has been established (Nguyen et al., 2024). Its infection has been reported related to BQCV and DWV (Abou Kubaa et al., 2018; Hartmann et al., 2015). We detected AmFV from all collection sites, while its viral loads were from extremely low at conventional farm C1 to medium-high at conventional farm C3. The Hubei partiti-like virus 34 was first discovered as an arthropod RNA virus (Shi et al., 2016) and was reported associated with hymenopteran species, including *A. mellifera* and Asian hornet (*Vespa velutina*) in East Asia and South Europe (Kwon et al., 2023; Marzoli et al., 2021). The current study reported the low to moderate infection of Hubei partiti-like virus 34 from all sites except conventional farm C3. The potential impact of this virus on honey bee health is still unknown.

The primary hosts for iflaviruses are arthropods (Mattia et al., 2025). We discovered two closely related novel ifla-like viruses. Unknown iflavirus 1 was detected in both *A. mellifera* and *C. calcarata*, while the unknown iflavirus 2 was detected only in *C. calcarata*. The prevalence of both viruses was low, however, the viral load of unknown iflavirus 2 was slightly higher in *C. calcarata* samples collected from conventional farm C1 and roadside site 1, compared to the unknown iflavirus 1 in the same samples.

The EPVs are double-stranded DNA (dsDNA) viruses that infect insects and predominantly insect pathogens. The unknown entomopoxivirus 1 detected in *C. calcarata* are more closely related to Yalta viruses and DIEPV than other entomopathogenic viruses. Although the role of Yalta virus is still unknown, the DIEPV has been found replicated in both parasitoid wasp *D. longicaudata* and its host fruit fly (*Anastrepha suspensa)*. Replication of this virus is highly virulent to the host fruit fly but has no negative impact on wasp host, suggesting a mutualistic symbiotic relationship between DIEPV and the parasitoid wasp (Coffman and Burke, 2020). Although both *C. calcarata* and *D. longicaudata* are from order Hymenoptera, *C. calcarata* is a solitary bee and does not share a parasitoid lifestyle. Therefore, unlike in *D. longicaudata*, there is no parasitoid-host relationship to explain the presence of the virus. Whether this putative novel entomopoxivirus primary infects *C. calcarata* or represents a spillover from another host remains to be determined.

Although our findings reveal the presence of viral sequences in both bee species, it does not confirm active infection or pathogenicity in these bees. Some of the viral sequences may originate from environmental exposure, gut contents, or external body surfaces, rather than systemic infection. Therefore, the present study does not infer a direct relationship between viral presence and bee health or stress levels (Olgun et al., 2020).

Many plant viruses can travel within or attach to pollen grains (Card et al., 2007; Fetters and Ashman, 2023), including BRCV, TSV, PSV, WCCV-1, WCCV-2, TRSV, ToRSV, and WCIMV as observed in the present study. However, only TSV, TRSV, and ToRSV have been proven vertically and horizontally transmitted by pollen. The rest were only detected by RNA-Seq (Fetters and Ashman, 2023). These findings show that pollinators such as *A. mellifera* posing potential risks due to these viruses. However, none of the detected viruses were on the list of known honey bee-transmitted viruses (Fetters and Ashman, 2023). TRSV has been reported to infect honey bees (Li et al., 2014); however, whether cross-kingdom infection facilitates diseases transmission remains unknown. On the other hand, bees are a powerful tool for monitoring plant diseases (Smadi et al., 2024). The plant viruses detected in *A. mellifera* resembles to the results of other metagenomic studies of bees and pollen at family and order levels (Fetters et al., 2022; Smadi et al., 2024), while the species composition is determined by plant and prevalence of plant viruses at the location.

Compared to *A. mellifera*, plant viruses in *C. calcarata* are less diverse. Previous studies have shown that pollen from clover and rose is a major food resource for bees (Lawson et al., 2016), and white clover pollen significantly increases the fitness of *C. calcarata* (Lawson et al., 2021). Consistent with that, among four plant viruses detected in *C. calcarata* virome, WCCV-1, WCCV-2, WCIMV primarily infect white clover (*Trifolium repens*), and the host range of ToRSV include rose and red clover (*T. pratense*). None of the detected viruses originated beyond *C. calcarata's* foraging range, suggesting that *C. calcarata* may be an ideal bee species for monitoring white clover-associated pathogens within localized areas.

The spillover of managed honey bee pathogen to wild bees has been considered as a threat to native bee populations (Fleites-Ayil et al., 2023; Nanetti et al., 2021). In genus *Ceratina*, DWV was found spillover to two species *C. dupla* (Singh et al., 2010) and *C. smaragdula* (Santamaria et al., 2018). The current study found that BQCV was present in both *A. mellifera* and *C. calcarata*, while DWV was detected only in *A. mellifera*. BQCV was only detected from conventional farm C2 in relatively low prevalence and viral load, indicating that this spillover to *C. calcarata* is not a common scenario.

Our results demonstrate that the distribution and abundance of viruses in bees were habitat-specific and varied across different landscapes. In *A. mellifera*, common bee-associated viruses such as BQCV, DWV, LSVs, SBV and AmFV were consistently present across sites, suggesting that these viromes communities are unaffected by agricultural practices. However, some other viruses such as LSV3 and Hubei partiti-like virus demonstrated differences between landscape patterns as higher abundance in organic farms compared to conventional farms and roadside habitats. This finding shows that organic habitats may influence viral dynamics, possibly through floral resources or pathogens exposure (Maurer et al., 2024; Temmermans et al., 2025). In contrast, the virome of C. *calcarata* was more restricted and habitat-dependent, with several viruses absent from organic farms. This shows viral diversity may be associated with species-specific and related to exposure, susceptibility or foraging behavior of different bee species. These results indicate that agricultural practices may affect the viromes communities in *A. mellifera* and C. *calcarata*. These differences were subtle compared to the strong host-level effects, but they suggest that local landscape context may still influence the prevalence of certain viruses, potentially through variation in floral resources, management practices, and surrounding pollinator communities. This information will be valuable for future work investigating environmental drivers of pollinator viromes.

In summary, this study provides comparative assessments of the viromes composition in managed honey bees and wild small carpenter bees across different landscape patterns. The results show that *A. mellifera* harbors a border diversity of both insect and plant-associated viruses than *C. calcarata*, suggesting that managed bees may play a critical role in virus maintenance and transmission in agroecosystems. Furthermore, the present study also shows the viral presence and abundance vary across agricultural landscapes, suggesting that environmental context influences virus-host dynamics in addition to species identity. These findings demonstrate that host identity and landscape context play important roles in shaping pollinator viromes. Future studies focusing viral prevalence to bee health outcomes, ecological interactions, and cross-specific transmission may help to understand the implications for pollinator conservation and agricultural sustainability.

Declarations

ACKNOWLEDGEMENTS

We are grateful for the help received from Stephanie Gardner, Katherine Odanaka, and Wyatt Shell with collecting bee samples. We thank the Genomic Shared Resource Shared Resource at the Ohio State University Comprehensive Cancer Center, Columbus, OH for generating the virus RNA-seq data reported in this publication.

Funding Declaration

Research reported in this publication was supported by USDA Evans Allen Fund NI231445XXXXG004, NI241445XXXXG004 for HL-B, ZZ, and RP, USDA NIFA awards Capacity Building Grant 2021-38821-34576 and 2020-67014-31557 for HL-B, and the NSF Louis Stokes Alliances for Minority Participation (LSAMP) program Ohio Alliance scholarship for JM.

DATA AVAILABILITY STATEMENT

The raw reads of RNA-Seq related to this study are available in NCBI BioProject ID PRJNA1308597.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

HL-B and SMR designed the experiment. SMR conducted the field studies and provided the samples. MCF and JM prepared samples for RNA-seq. ZZ conducted data analysis and wrote the first draft of the manuscript. RP revised and finalized the manuscript. All authors reviewed the final manuscript.

CONFLICTOF INTEREST STATEMENT

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.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this study are available in the online version of this paper.

References

1. Abou Kubaa, R., Molinatto, G., Solaiman Khaled, B., Daher-Hjaij, N., Heinoun, K. & Saponari, M.(2018) First detection of black queen cell virus, *Varroa destructor* macula-like virus, *Apis mellifera* filamentous virus and *Nosema ceranae* in Syrian honey bees *Apis mellifera syriaca*. *Bulletin of Insectology*, 71(2), 217–224. Available from: https://api.semanticscholar.org/CorpusID:201735642
2. Bolger, A.M., Lohse, M. & Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114-2120. Available from: https://doi.org/10.1093/bioinformatics/btu170.
3. Breeze, T.D., Bailey, A.P., Balcombe, K.G. & Potts, S.G. (2011) Pollination services in the UK: How important are honeybees? *Agriculture, Ecosystems & Environment*, 142(3), 137-143. Available from: https://doi.org/10.1016/j.agee.2011.03.020.
4. Brittain, C., Kremen, C. & Klein, A.-M. (2013) Biodiversity buffers pollination from changes in environmental conditions. *Global Change Biology*, 19(2), 540-547. Available from: https://doi.org/10.1111/gcb.12043.
5. Brown, M.J.F., Dicks, L.V., Paxton, R.J., Baldock, K.C.R., Barron, A.B. et al. (2016) A horizon scan of future threats and opportunities for pollinators and pollination. *PeerJ*, 4, e2249. Available from: https://doi.org/10.7717/peerj.2249.
6. Card, S.D., Pearson, M.N. & Clover, G.R.G. (2007) Plant pathogens transmitted by pollen. Australas. *Plant Pathology*, 36(5), 455-461. Available from: https://doi.org/10.1071/AP07050.
7. Chen, N.-C., Wang, C.-H., Yoshimura, M., Yeh, Y.-Q., Guan, H.-H., Chuankhayan, P., Lin, C.C., Lin, P.J., Huang, Y.C., Wakatsuki, S., Ho, M.C. & Chen, C.-J. (2023) Structures of honeybee-infecting Lake Sinai virus reveal domain functions and capsid assembly with dynamic motions. *Nature Communications*, 14(1), 545. Available from: https://doi.org/10.1038/s41467-023-36235-3.
8. Chen, Y., Lun, A.T. & Smyth, G.K. (2016) From reads to genes to pathways: differential expression analysis of RNA-Seq experiments using Rsubread and the edgeR quasi-likelihood pipeline. *F1000Research*, 5, 1438. Available from: https://doi.org/10.12688/f1000research.8987.2.
9. Coffman, K.A. & Burke, G.R. (2020) Genomic analysis reveals an exogenous viral symbiont with dual functionality in parasitoid wasps and their hosts. *PLoS Pathology*, 16(11), e1009069. Available from: https://doi.org/10.1371/journal.ppat.1009069.
10. Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N. A. et al. (2007) A metagenomic survey of microbes in honey bee colony collapse disorder. *Science*, 318(5848), 283-287. Available from: https://doi.org/doi:10.1126/science.1146498.
11. Dalmon, A., Diévart, V., Thomasson, M., Fouque, R., Vaissière, B.E., Guilbaud, L., Le Conte, Y. & Henry, M. (2021) Possible spillover of pathogens between bee communities foraging on the same floral resource. *Insects*, 12(2), 122. Available from: https://doi.org/10.3390/insects12020122.
12. Daughenbaugh, K.F., Martin, M., Brutscher, L.M., Cavigli, I., Garcia, E., Lavin, M. & Flenniken, M.L. (2015) Honey bee infecting Lake Sinai viruses. *Viruses*, 7(6), 3285-3309. Available from: https://doi.org/10.3390/v7062772.
13. Deutsch, K.R., Graham, J.R., Boncristiani, H.F., Bustamante, T., Mortensen, A.N., Schmehl, D.R., Wedde, A.E., Lopez, D.L., Evans, J.D. & Ellis, J.D. (2023) Widespread distribution of honey bee-associated pathogens in native bees and wasps: Trends in pathogen prevalence and co-occurrence. *Journal of Invertebrate Pathology*, 200, 107973. Available from: https://doi.org/10.1016/j.jip.2023.107973.
14. Dolezal, A.G., Hendrix, S.D., Scavo, N.A., Carrillo-Tripp, J., Harris, M.A., Wheelock, M. J., O'Neal, M.E. & Toth, A.L. (2016) Honey bee viruses in wild bees: viral prevalence, loads, and experimental Inoculation. *PLoS ONE*, 11(11), e0166190. Available from: https://doi.org/10.1371/journal.pone.0166190.
15. Doublet, V., Doyle, T., Refoy, I., Hedges, S., Carvell, C., Brown, M.J.F. & Wilfert, L. (2022) Increasing flower species richness in agricultural landscapes alters insect pollinator networks: Implications for bee health and competition. Ecology and Evolution,12(10), e9442. Available from: https://doi.org/10.1002/ece3.9442.
16. Drossart, M. & Gérard, M. (2020) Beyond the decline of wild bees: optimizing conservation measures and bringing together the actors. *Insects*, 11(9), 649. Available from: https://www.mdpi.com/2075-4450/11/9/649.

17. Drummond, F.A., Lund, J. & Eitzer, B. (2021) Honey bee health in Maine wild blueberry production. *Insects*, 12(6), 523. Available from: https://doi.org/10.3390/insects12060523.
18. Fetters, A.M. & Ashman, T.-L. (2023) The pollen virome: A review of pollen-associated viruses and consequences for plants and their interactions with pollinators. *American Journal of Botany*, 110(6), e16144. Available from: https://doi.org/10.1002/ajb2.16144.
19. Fetters, A.M., Cantalupo, P.G., Wei, N., Robles, M.T.S., Stanley, A., Stephens, J.D., Pipas, J.M. & Ashman, T.-L. (2022) The pollen virome of wild plants and its association with variation in floral traits and land use. *Nature Communications*, 13(1), 523. Available from: https://doi.org/10.1038/s41467-022-28143-9.
20. Fleites-Ayil, F.A., Medina-Medina, L.A., Quezada Euán, J.J.G., Stolle, E., Theodorou, P., Tragust, S. & Paxton, R.J. (2023) Trouble in the tropics: Pathogen spillover is a threat for native stingless bees. *Biological Conservation*, 284, 110150. Available from: https://doi.org/10.1016/j.biocon.2023.110150.
21. Goulson, D., Nicholls, E., Botías, C. & Rotheray, E.L. (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, 347(6229), 1255957. Available from: https://doi.org/10.1126/science.1255957.
22. Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A. et al. (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology*, (7), 644-652. Available from: http://dx.doi.org/10.1038/nbt.1883.
23. Guo, Y., Ma, C., Zhao, W., Kuang, H., Tian, Y., Zhang, H., Xue, Y., Li-Byarlay, H., Dong, K. & Gong, X. (2025) Chronic exposure to field-level thiamethoxam impairs gut tissue and reduces honeybee (*Apis cerana*) survival. *Insects*, 16(4), p.372. Available from: http://dx.doi.org/10.3390/insects16040372.
24. Hartmann, U., Forsgren, E., Charrière, J.-D., Neumann, P. & Gauthier, L. (2015) Dynamics of *Apis mellifera* filamentous virus (AmFV) infections in honey bees and relationships with other parasites. *Viruses*, 7(5), 2654-2667. Available from: https://doi.org/10.3390/v7052654.
25. Katumo, D.M., Liang, H., Ochola, A.C., Lv, M., Wang, Q.-F. & Yang, C.-F. (2022) Pollinator diversity benefits natural and agricultural ecosystems, environmental health, and human welfare. *Plant Diversity*, 44(5), 429-435. Available from: https://doi.org/10.1016/j.pld.2022.01.005.
26. Khalifa, S.A.M., Elshafiey, E.H., Shetaia, A.A., El-Wahed, A.A.A., Algethami, A.F. et al. (2021) Overview of bee pollination and its economic value for crop production. *Insects*, 12(8), 688. Available from: https://www.mdpi.com/2075-4450/12/8/688.
27. Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. & Tscharntke, T. (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences*, 274(1608), 303-313. Available from: https://doi.org/10.1098/rspb.2006.3721.
28. Kwon, M., Jung, C. & Kil, E.-J. (2023) Metagenomic analysis of viromes in honey bee colonies (*Apis mellifera*; Hymenoptera: Apidae) after mass disappearance in Korea. *Frontiers in Cellular and Infection Microbiology*, 13, 1124596. Available from: https://doi.org/10.3389/fcimb.2023.1124596.
29. Langmead, B. & Salzberg, S.L. (2012) Fast gapped-read alignment with Bowtie 2. *Nature Methods*, 9(4), 357-359. Available from: https://doi.org/10.1038/nmeth.1923.
30. Lawson, S.P., Ciaccio, K.N. & Rehan, S.M. (2016) Maternal manipulation of pollen provisions affects worker production in a small carpenter bee. *Behavioral Ecology and Sociobiology*,70(11), 1891-1900. Available from: https://doi.org/10.1007/s00265-016-2194-z.
31. Lawson, S.P., Helmreich, S.L. & Rehan, S.M. (2017) Effects of nutritional deprivation on development and behavior in the subsocial bee *Ceratina calcarata* (Hymenoptera: Xylocopinae). *Journal of Experimental Biology*, 220(23), 4456-4462. Available from: https://doi.org/10.1242/jeb.160531.
32. Lawson, S.P., Kennedy, K.B. & Rehan, S.M. (2021) Pollen composition significantly impacts the development and survival of the native small carpenter bee, *Ceratina calcarata*. *Ecological Entomology*, 46(2), 232-239. Available from: https://doi.org/10.1111/een.12955.
33. Li, J.L., Cornman, R.S., Evans Jay, D., Pettis Jeffery, S., Zhao, Y., Murphy, C., Peng, W.J., Wu, J., Hamilton, M., Boncristiani, H.F.Jr., Zhou, L., Hammond, J. & Chen, P.Y. (2014) Systemic spread and propagation of a plant-pathogenic virus in European honeybees, *Apis mellifera*. *mBio*, 5(1), e00898-13. Available from: https://doi.org/10.1128/mbio.00898-13.
34. Li-Byarlay, H., Boncristiani, H., Howell, G., Herman, J., Clark, L., Strand, M.K., Tarpy, D. & Rueppell, O. (2020) Transcriptomic and epigenomic dynamics of honey bees in response to lethal viral infection. *Frontiers in Genetics*, 11, p.566320. Available from: https://doi.org/10.3389/fgene.2020.566320.

35. Liao, Y., Smyth, G.K. & Shi, W. (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, 30(7), 923-930. Available from: https://doi.org/10.1093/bioinformatics/btt656.
36. Marzoli, F., Forzan, M., Bortolotti, L., Pacini, M.I., Rodríguez-Flores, M.S., Felicioli, A. & Mazzei, M. (2021) Next generation sequencing study on RNA viruses of *Vespa velutina* and *Apis mellifera* sharing the same foraging area. *Transboundary and Emerging Diseases*,68(4), 2261-2273. Available from: https://doi.org/10.1111/tbed.13878.
37. Mattia, A., Huditz, H.I., van Oers, M.M. & Ros, V.I.D. (2025) Iflaviruses in arthropods: when small is mighty. *Insect Science*, Available from: https://doi.org/10.1111/1744-7917.70177
38. Maurer, C., Schauer, A., Yañez, O., Neumann, P., Gajda, A., Paxton, R.J., Pellissier, L., Schweiger, O., Szentgyörgyi, H., Vanbergen, A.J. & Albrecht, M. (2024) Species traits, landscape quality and floral resource overlap with honeybees determine virus transmission in plant-pollinator networks. *Nature Ecology & Evolution*, 8(12), 2239–2251. Available from: https://doi.org/10.1038/s41559-024-02555-w
39. Nanetti, A., Bortolotti, L., & Cilia, G. (2021) Pathogens spillover from honey bees to other arthropods. *Pathogens*, 10(8), 1044. Available from: https://doi.org/10.3390/pathogens10081044.
40. Nguyen, T.T., Yoo, M.S., Lee, H.S., Truong, A.T., Youn, S.Y., Lee, S.J., Kim, J. & Cho, Y.S. (2024) First detection and prevalence of *Apis mellifera* filamentous virus in *Apis mellifera* and *Varroa destructor* in the Republic of Korea. *Scientific Report*, 14, 14105. Available from: https://doi.org/10.1038/s41598-024-64882-z
41. Nurk, S., Meleshko, D., Korobeynikov, A. & Pevzner, P.A. (2017) metaSPAdes: a new versatile metagenomic assembler. *Genome Research*, 27(5), 824-834. Available from: https://doi.org/10.1101/gr.213959.116.
42. Olgun, T., Everhart, S.E., Anderson, T. & Wu-Smart, J. (2020) Comparative analysis of viruses in four bee species collected from agricultural, urban, and natural landscapes. *PLoS One*. 15(6):e0234431. Available from: https://doi.org/10.1371/journal.pone.0234431.
43. Ollerton, J. (2017) Pollinator diversity: distribution, ecological function, and conservation. *Annual Review of Ecology, Evolution, and Systematics*, 48, 353-376. Available from: https://doi.org/10.1146/annurev-ecolsys-110316-022919.
44. Ollerton, J., Winfree, R. & Tarrant, S. (2011) How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321-326. Available from: https://doi.org/10.1111/j.1600-0706.2010.18644.x.
45. Piot, N., Schweiger, O., Meeus, I., Yañez, O., Straub, L. et al. (2022) Honey bees and climate explain viral prevalence in wild bee communities on a continental scale. Scientific Report, 12, 1904. Available from: https://doi.org/10.1038/s41598-022-05603-2.
46. Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O. & Kunin, W.E. (2010) Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, 25(6), 345-353. Available from: https://doi.org/10.1016/j.tree.2010.01.007.
47. Proesmans, W., Albrecht, M., Gajda, A., Neumann, P., Paxton, R. J., Pioz, M., Polzin, C., Schweiger, O., Settele, J., Szentgyörgyi, H., Thulke, H. H. & Vanbergen, A. J. (2021) Pathways for novel epidemiology: plant-pollinator-pathogen networks and global change. *Trends in Ecology & Evolution*, 36(7), 623–636. Available from: https://doi.org/10.1016/j.tree.2021.03.006.
48. Rehan, S.M., Glastad, K.M., Lawson, S.P. & Hunt, B.G. (2016) The genome and methylome of a subsocial small carpenter bee, *Ceratina calcarata*. *Genome Biology and Evolution*, 8(5), 1401-1410. Available from: https://doi.org/10.1093/gbe/evw079.
49. Rehan, S.M. & Richards, M.H. (2010) Nesting biology and subsociality in *Ceratina calcarata* (Hymenoptera: Apidae). *The Canadian Entomologist*, 142(1), 65-74. Available from: https://doi.org/10.4039/n09-056.
50. Roberts, J.M.K., Anderson, D.L. & Durr, P.A. (2018) Metagenomic analysis of Varroa-free Australian honey bees (*Apis mellifera*) shows a diverse Picornavirales virome. *Journal of General Virology*, 99(6), 818-826. Available from: https://doi.org/10.1099/jgv.0.001073.
51. Robinson, M.D., McCarthy, D.J. & Smyth, G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, 26(1), 139-140. Available from: https://doi.org/10.1093/bioinformatics/btp616.
52. Roulston, T.H. & Goodell, K. (2010) The role of resources and risks in regulating wild bee populations. *Annual Review of Entomology*, 56(1), 293-312. Available from: https://doi.org/10.1146/annurev-ento-120709-144802.
53. Rowhani, A., Daubert, S.D., Uyemoto, J.K., Al Rwahnih, M. & Fuchs, M. (2017) American Nepoviruses. In: Meng, B., Martelli, G.P., Golino, D.A., Fuchs, M., (Eds.), Grapevine viruses: molecular biology, diagnostics and management. Springer International Publishing, Gewerbestrasse 11, 6330 Cham, Switzerland. pp. 109-126. Available from: https://doi.org/10.1007/978-3-319-57706-7_5.

54. Santamaria, J., Villalobos, E.M., Brettell, L.E., Nikaido, S., Graham, J.R. & Martin, S. (2018) Evidence of *Varroa*-mediated deformed wing virus spillover in Hawaii. *Journal of Invertebrate Pathology*, 151, 126-130. Available from: https://doi.org/10.1016/j.jip.2017.11.008
55. Seitz, K., Buczolich, K., Dikunová, A., Plevka, P., Power, K., Rümenapf, T. & Lamp, B. (2019) A molecular clone of Chronic Bee Paralysis Virus (CBPV) causes mortality in honey bee pupae (*Apis mellifera*). *Scientific Report*, 9(1), 16274. Available from: https://doi.org/10.1038/s41598-019-52822-1.
56. Shell, W.A. & Rehan, S.M. (2017) The price of insurance: costs and benefits of worker production in a facultatively social bee. *Behavioural Ecology*, 29(1), 204-211. Available from: https://doi.org/10.1093/beheco/arx146.
57. Shi, M., Lin, X.-D., Tian, J.-H., Chen, L.-J., Chen, X., Li, C.-X., Qin, X.C., Li, J., Cao, J.P., Eden, J.S., Buchmann, J., Wang, W., Xu, J., Holmes, E.C. & Zhang, Y.-Z. (2016) Redefining the invertebrate RNA virosphere. *Nature*, 540(7634), 539-543. Available from: https://doi.org/10.1038/nature20167.
58. Šimenc, L., Kuhar, U., Jamnikar-Ciglenečki, U. & Toplak, I. (2020) First complete genome of Lake Sinai virus lineage 3 and genetic diversity of Lake Sinai virus strains from honey bees and bumble bees. *Journal of Economic Entomology*, 113(3), 1055-1061. Available from: https://doi.org/10.1093/jee/toaa049.
59. Singh, R., Levitt, A.L., Rajotte, E.G., Holmes, E.C., Ostiguy, N., vanEngelsdorp, D., Lipkin, W.I., Depamphilis, C.W., Toth, A.L. & Cox-Foster, D.L. (2010) RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-apis hymenopteran species. *PLoS ONE*, 5(12), e14357. Available from: https://doi.org/10.1371/journal.pone.0014357.
60. Siviter, H., Fisher, A., Baer, B., Brown, M.J.F., Camargo, I.F., et al. (2023) Protecting pollinators and our food supply: understanding and managing threats to pollinator health. *Insectes Sociaux*, 70(1), 5-16. Available from: https://doi.org/10.1007/s00040-022-00897-x.
61. Smadi, M., Lee, E., Phelan, J., Wang, A., Bilodeau, G.J., Pernal, S.F., Guarna, M.M., Rott, M. & Griffiths, J.S. (2024) Plant virus diversity in bee and pollen samples from apple (*Malus domestica*) and sweet cherry (*Prunus avium*) agroecosystems. Frontiers in Plant Science, 15, 1335281. Available from: https://doi.org/10.3389/fpls.2024.1335281.
62. Susi, H. & Laine, A.L. (2021) Agricultural land use disrupts biodiversity mediation of virus infections in wild plant populations. *New Phytologist* 230(6), 2447-2458. Available from: https://doi.org/10.1111/nph.17156.
63. Tamura, K., Stecher, G. & Kumar, S. (2021) MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7), 3022-3027. Available from: https://doi.org/10.1093/molbev/msab120.
64. Tehel, A., Brown, M.J.F. & Paxton, R.J. (2016) Impact of managed honey bee viruses on wild bees. *Current Opinion in Virology*, 19, 16-22. Available from: https://doi.org/10.1016/j.coviro.2016.06.006.
65. Temmermans, J., Legein, M., Checchia, I., Felis, G.E., Smets, W., Karise, R. & Lebeer, S. (2025) Agricultural practices and pollinators modulate the anthosphere microbiome. *ISME Communications*, 5(1), ycaf026. Available from: https://doi.org/10.1093/ismeco/ycaf026
66. Toplak, I., Šimenc, L., Pislak Ocepek, M. & Bevk, D. (2020) Determination of genetically identical strains of four honeybee viruses in bumblebee positive samples. *Viruses*, 12(11), 1310. Available from: https://doi.org/10.3390/v12111310
67. Tucker, E.M. & Rehan, S.M. (2017) Wild bee community assemblages across agricultural landscapes. *Journal of Agricultural and Urban Entomology*, 33(1), 77-104. Available from: https://doi.org/10.3954/1523-5475-33.1.77.
68. Tucker, E.M. & Rehan, S.M. (2018) Farming for bees: annual variation in pollinator populations across agricultural landscapes. *Agricultural and Forest Entomology*, 20(4), 541-548. Available from: https://doi.org/10.1111/afe.12287.
69. Ullah, A., Tlak Gajger, I., Majoros, A., Dar, S.A., Khan, S., Kalimullah, Haleem Shah, A., Nasir Khabir, M., Hussain, R., Khan, H.U., Hameed, M. & Anjum, S.I. (2021) Viral impacts on honey bee populations: A review. *Saudi Journal of Biological Sciences*, 28(1):523-530. Available from: https://doi.org/10.1016/j.sjbs.2020.10.037.
70. Walker, P.J., Siddell, S.G., Lefkowitz, E.J., Mushegian, A.R., Adriaenssens, E.M., Alfenas-Zerbini, P. et al. (2022) Recent changes to virus taxonomy ratified by the International Committee on Taxonomy of Viruses. *Archives of Virology*,167(11), 2429-2440. Available from: https://doi.org/10.1007/s00705-022-05516-5.
71. Winfree, R., Williams, N.M., Dushoff, J. & Kremen, C. (2007) Native bees provide insurance against ongoing honey bee losses. *Ecology Letters*, 10(11), 1105-1113. Available from: https://doi.org/10.1111/j.1461-0248.2007.01110.x.

72. Zhang, X., He, S.Y., Evans, J.D., Pettis, J.S., Yin, G.F. & Chen, Y.P. (2012) New evidence that deformed wing virus and black queen cell virus are multi-host pathogens. *Journal of Invertebrate Pathology*, 109(1), 156-159. Available from: https://doi.org/10.1016/j.jip.2011.09.010.

Figures

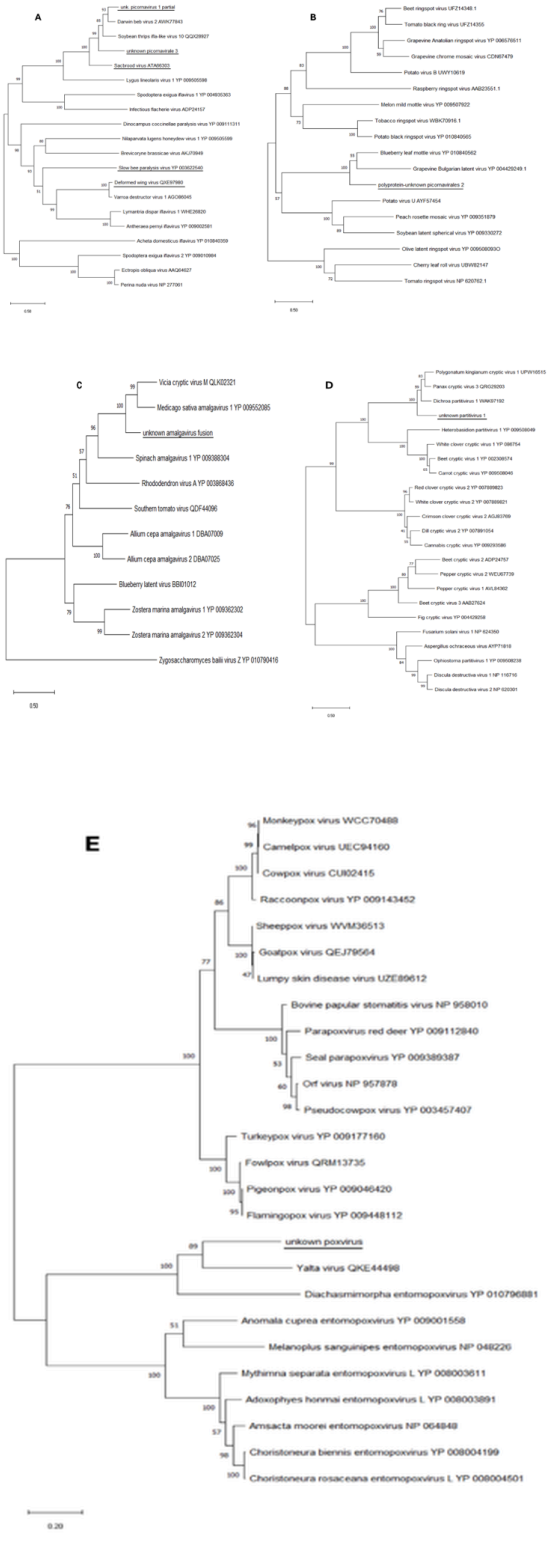


Figure 1

Phylogenetic trees of putative novel viruses discovered from *A. mellifera* and *C. calcarata* virome. Trees were constructed using maximum-likelihood analysis of viral protein sequences: **(A)** polyprotein (Iflavirus), **(B)**polyprotein from RNA2 (Neprovirus), **(C)** RNA-dependent RNA polymerase (Amalgavirus), **(D)** RNA-dependent RNA polymerase from RNA1 (Partitivirus), and **(E)**RNA-dependent RNA polymerase submit RPO132 (Poxvirus). Branch labels indicate virus family or closest reference sequence, and viruses identified in this study are underlined. Abbreviations and protein accessions of known viruses are included in **Supplementary File S2.** Scale bars indicate the number of amino acid substitutions per site. These phylogenies illustrate evolutionary relationships and do not reflect relative abundance

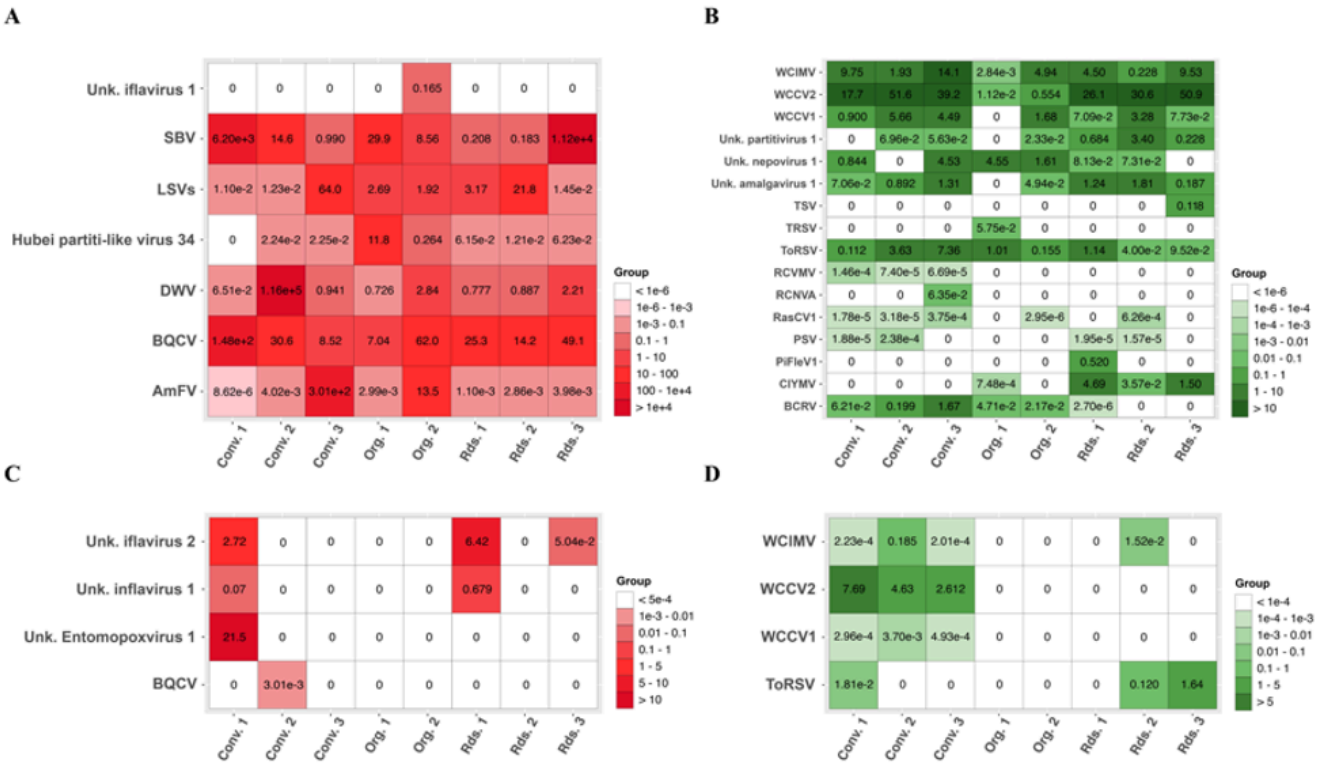


Figure 2

Heatmaps demonstrating the relative abundance of **(A)** insect viruses in *A. mellifera*, **(B)** plant viruses in *A. mellifera*, **(C)** insect viruses in C. *calcarata*, and **(D)** plant viruses in *C. calcarata* at each sampling site. Average normalized count-per-million reads (cpm) from RNA-Seq was calculated for each virus of each site and used in the heatmap. White color indicates the virus is undetected on that site. Potential insect and plant viruses were included.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- S1sampleinforation.xlsx
- S2Phylogentictreeinfo.xlsx