



Virome analysis of *Capsicum* spp. and the potential aphid vector *Aphis gossypii* through next-generation sequencing

Diana Nataly Duque-Gamboa^{1,2} · Nelson Toro-Perea^{1,2}

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Abstract

Chili peppers (*Capsicum* spp.), an important export crop for the agroindustry of southwestern Colombia, face phytosanitary problems that are presumably attributable to viral infections. To date, virus detection in these crops has primarily relied on ELISA, PCR, and RT-PCR techniques. To characterize the virome of commercial *Capsicum* spp. crops and identify potential virus vectors, an ecogenomic analysis was conducted using total RNA extracted from symptomatic leaf tissues of cayenne pepper (*Capsicum annuum*) and tabasco pepper (*Capsicum frutescens*), as well as from the dominant aphid species present in the cultivated area of the inter-Andean valley of the Cauca River, Colombia. Analysis of the RNA libraries from *C. annuum* and *C. frutescens* revealed the presence of cryptic viruses, including bell pepper endornavirus (BPEV; *Alphaendornavirus capsici*), pepper cryptic virus 1 (PCV1; *Deltapartitivirus unocapsici*), and pepper cryptic virus 2 (PCV2; *Deltapartitivirus duocapsici*); as well as symptomatic viruses transmitted by vectors, such as cucumber mosaic virus (CMV; *Cucumovirus CMV*), pepper vein yellows virus (PeVYV; *Polerovirus PEVYV*), and pepper severe mottle virus (PepSMoV); and the symptomatic, mechanically transmitted pepper mild mottle virus (PMMoV; *Tobamovirus capsici*). The RNA library from *A. gossypii* revealed the presence of CMV and PEVYV. These results suggest that *A. gossypii*, which often occurs at high population densities in commercial crops, may serve as a vector transmitting these symptomatic viruses. The current study presents the first report of mechanically transmitted symptomatic viruses from Colombia.

Keywords Chili pepper virome · Aphid virome · RNA viruses · Plant viruses · NGS

Introduction

Capsicum spp. cultivation in Colombia is limited by several diseases caused by pathogens, including Oomycetes such as *Phytophthora capsici* Leonian (Cardona 2013), and viruses belonging to the *Bromoviridae* and *Potyviridae* families (Guaiteiro-Díaz 2011; Tamayo 2005). The aphid species *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover (Hemiptera: Aphididae) are reported as pests in chili pepper crops in the inter-andean valley of the Cauca river (Duque-Gamboa et al. 2021; Tálaga-Taquinas et al. 2020). They have already been characterized as competent vectors for

the transmission of *Bromoviridae* and *Potyviridae* viruses in several studies conducted in Brazil and the United States (Brault et al. 2010; Gildow et al. 2008; Pinto et al. 2008a, b). However, in Colombia, information about these species acting as virus vectors in chili pepper is still missing. In the absence of this information, current disease management practices have primarily focused on eradicating presumed insect vectors thought to transmit phytopathogenic viruses. In Colombia, investigations aimed at characterizing plant viral diseases in pepper crops have primarily relied on methods such as ELISA, PCR, and RT-PCR for virus detection, following the identification of symptomatic plants. These studies have focused on viruses such as pepper rugose mosaic virus (PRMV, *Geminiviridae*), cucumber mosaic virus (CMV, *Bromoviridae*), pepper severe mottle virus (PepSMoV), and pepper deforming mosaic virus (PepDMV, *Potyviridae*) (García and Pardey 2009; Morales et al. 2005; Rivera-Toro et al. 2020, 2021; Tamayo 2005; Tamayo and Jaramillo 2006). However, these diagnostic methods require prior knowledge of the target viruses' identities in order to design specific primers or probes for detection, which can

✉ Diana Nataly Duque-Gamboa
diana.nataly.duque@correounivalle.edu.co

¹ Department of Biology, Genetics Section, Universidad del Valle, Calle 13 # 100-00, Cali, Colombia

² Centro de Bioinformática y Fotonica-CIBioFi, Universidad del Valle, Calle 13 # 100-00, Cali, Colombia

be a limiting factor when attempting to characterize the complete plant virome, and may underestimate the diversity of viruses capable of infecting *Capsicum* spp. (Adams et al. 2009; Boonhama et al. 2014; Wylie et al. 2012).

Viruses constitute a polyphyletic group lacking a universal genomic region that can serve as a barcode, and they exhibit all possible forms of nucleic acids. Therefore, massive amplicon sequencing is not a feasible strategy for virome characterization (Roux et al. 2017). However, next-generation sequencing tools applied to the analysis of the RNA of a host organism or potential vector, using ecogenomic studies, can contribute without bias to the characterization of the diversity of viruses present (Boonhama et al. 2014; Roossinck 2010).

Ecogenomic studies differ from metagenomic studies, since the virome is analyzed directly in specific hosts, allowing inferences to be made about viral ecology, while in metagenomic studies, when studying environmental samples, the association of a virus to a host is not very feasible (Roossinck et al. 2010). In the last decade, plant ecogenomics has contributed to the identification of asymptomatic viruses, co-infections, vector–virus–plant associations, and the description of new viruses (Bejerman et al. 2020; Stobbe and Roossinck 2014; Wylie et al. 2012, 2014). Therefore, the objective of this study was to characterize, under field conditions, the virome of symptomatic *Capsicum* spp. plants and the most abundant aphid species in chili pepper crops (*Aphis gossypii*) in the inter-Andean valley of the Cauca River, Colombia, using next-generation sequencing (NGS) and an ecogenomic approach.

Materials and methods

Study area and sample collection

The inter-Andean valley of the Cauca River is a fertile depression situated between the Central and Western Cordilleras of the Colombian Andes (Fig. 1), ranging from approximately 900 to 1,200 m above sea level, and characterized by an extensive agricultural matrix embedded within a landscape of fragmented tropical dry forests (Alvarado-Solano and Otero 2015; IGAC 1998; Pizano and Garcia 2014).

Sampling was conducted in commercial crops of cayenne pepper (*Capsicum annuum* L.) and tabasco pepper (*Capsicum frutescens* L.) across seven locations in the inter-Andean valley of the Cauca River (Table 1; Fig. 1). Leaf samples were collected seven months after seedling transplanting, including both young and mature leaves showing viral infection symptoms such as dwarfing, shortened internodes, fruit deformation, leaf curling, and chlorosis affecting entire leaf blades or appearing as spots or mosaic

patterns. A total of 25 *C. annuum* and 40 *C. frutescens* plants were sampled. Leaves were placed in aluminum envelopes, with 5–7 leaves collected per *C. annuum* plant and 10–15 per *C. frutescens* plant.

In the field, symptomatic *Capsicum* plants were inspected for the presence of aphids, and colonies of *Aphis gossypii* were manually collected from all sampling sites. From each site, 20 colonies containing both adults and nymphs, were individually placed into 2-ml cryovials, yielding a total of 180 adult insects collected (Table 1).

At each sampling site, both plant and insect samples were carefully labeled and immediately frozen in liquid nitrogen. Upon arrival at the Molecular Biology Laboratory of the Universidad del Valle, they were stored at -80 °C until RNA extraction.

RNA extraction, library construction, and sequencing

RNA was extracted from the leaf tissue of individual *C. annuum* and *C. frutescens* plants with signs of virosis, as well as from a pooled sample 180 adult aphids collected across all sampling sites, using the commercial kit Total RNA Isolation System (Promega). For each extraction, tissue was ground in liquid nitrogen, and 34 mg of the resulting macerated material was used for subsequent RNA isolation.

Three libraries were constructed from pooled RNA samples of *C. annuum* (25 plants), pooled RNA samples of *C. frutescens* (40 plants), and the total RNA sample of *A. gossypii*. Three libraries were made for RNA sequencing on the Illumina HiSeq 2500 platform. The commercial kit TruSeq Stranded Total RNA with Ribo-Zero (Human/Mouse/Rat) was used to obtain the *A. gossypii* library. The construction of the *Capsicum* spp. libraries was performed with the TruSeq Stranded Total RNA with Ribo-Zero Plant kit. The library construction and sequencing process was performed through an external sequencing facility, MacroGen Inc. South Korea.

Bioinformatic data analysis

For each library, the quality of the readings was evaluated using FastQC (Andrews 2018), the filtering of the sequences was performed considering the quality (>Q30) and length (>100 bp) using Trimmomatic (Bolger et al. 2014). Subsequently, a de novo assembly was performed using Trinity (Grabherr et al. 2011; Haas et al. 2013). To assign the identity of the assembled contigs, a reference database of viral genomes was constructed using BLASTdb (Camacho 2017; Camacho and Madden 2023). BLAST+2.6.0 was used for the assignment and annotation of contigs to reference sequences, considering identity greater than 90%,

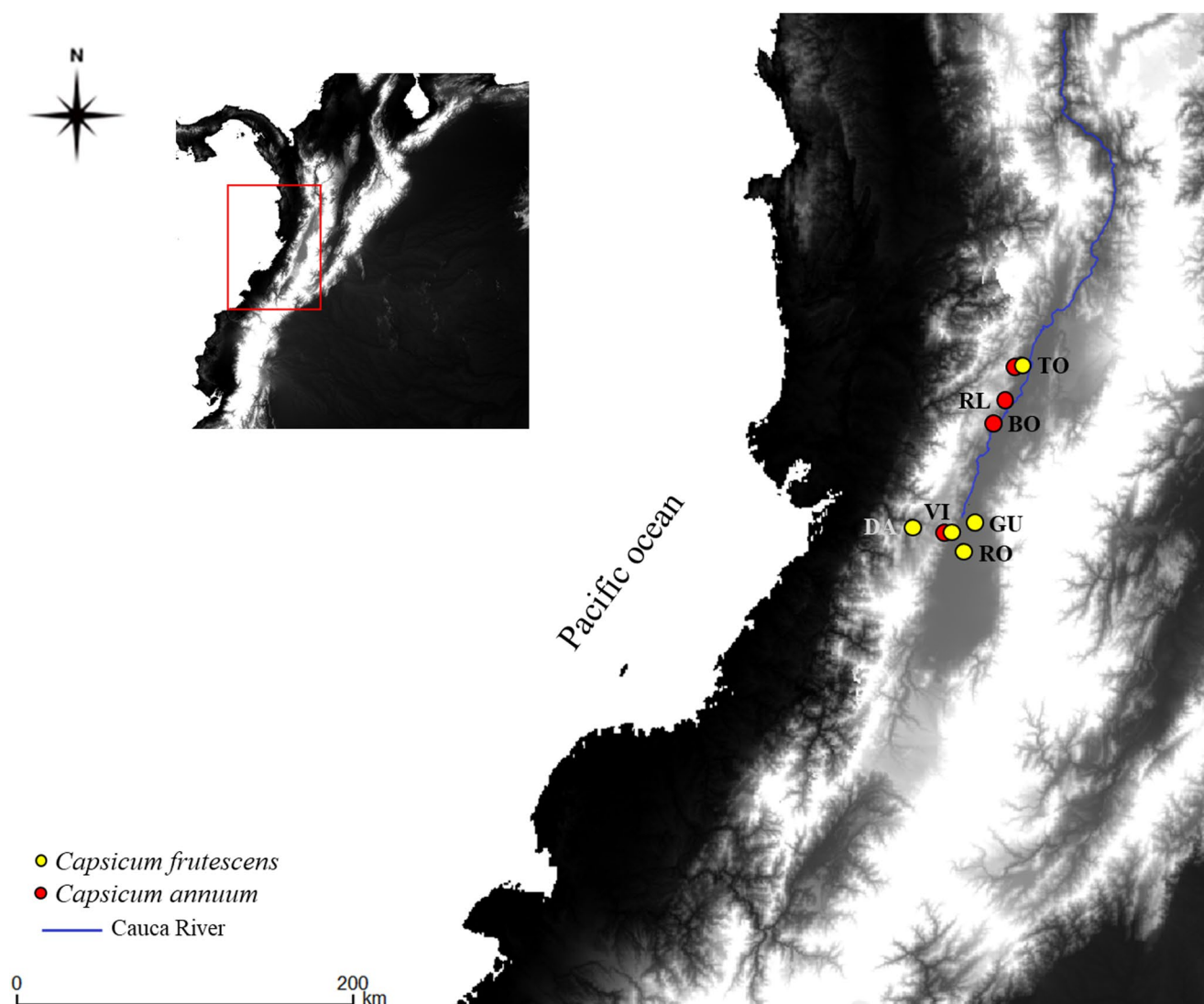


Fig. 1 Sampling sites of chili pepper crops, *C. annuum* and *C. frutescens*, and aphids, in the inter-Andean valley of the Cauca River, Colombia

Table 1 Sampling sites, aphid species and plant material collected

Sampling sites	Code	North	West	Pepper Crop	Plant species	Number of plants sampled*	Insect species	Number of adult insects sampled**
Rozo	RO	3.62	-76.39	Tabasco	<i>Capsicum frutescens</i>	10	<i>Aphis gossypii</i>	20
Bolívar	BO	4.30	-76.21	Cayenne	<i>Capsicum annuum</i>	8	<i>Aphis gossypii</i>	20
Vijes	VI	3.71	-76.43	Tabasco	<i>Capsicum frutescens</i>	10	<i>Aphis gossypii</i>	20
Vijes	VI	3.71	-76.43	Cayenne	<i>Capsicum annuum</i>	5	<i>Aphis gossypii</i>	20
Guacarí	GU	3.75	-76.33	Tabasco	<i>Capsicum frutescens</i>	9	<i>Aphis gossypii</i>	20
Roldanillo	RL	4.40	-76.15	Cayenne	<i>Capsicum annuum</i>	4	<i>Aphis gossypii</i>	20
Toro	TO	4.61	-76.08	Cayenne	<i>Capsicum annuum</i>	8	<i>Aphis gossypii</i>	20
Toro	TO	4.61	-76.08	Tabasco	<i>Capsicum frutescens</i>	6	<i>Aphis gossypii</i>	20
Dagua	DA	3.75	-76.66	Tabasco	<i>Capsicum frutescens</i>	5	<i>Aphis gossypii</i>	20

*Between 5 (cayenne pepper) and 15 (tabasco pepper) leaves were sampled for each plant. **Adults were collected from different colonies, winged adults were not found

coverage greater than 60%, and e-value less than 0.001 (Camacho 2017). In order to compare the viromes in terms of virus richness and abundance, a Hutcheson's t-test for diversity, based on the Bray-Curtis, Shannon (H) and Simpson index (D), was used with R (R Core Team 2018). Open reading frames (ORFs) and conserved domains/motifs were detected with the ORFfinder and Batch CD-Search tools (Marchler-Bauer et al. 2017; Marchler-Bauer and Bryant 2004). Sequence alignment and phylogenetic inference using the maximum likelihood method, were performed with the Molecular Evolutionary Genetics Analysis software MEGA X (Kumar et al. 2018).

Results

Virus composition

Approximately 50 million paired-end reads (101 bp) were obtained from each library and subsequently used for contig assembly. Of these, around 15 million viral reads were detected in *C. annuum*, 30 million in *C. frutescens*, and 170,099 reads were identified as viral phytopathogens in *A. gossypii*. The BLAST analysis of the virome of the aphid *A. gossypii* and the cultivated plants *C. annuum* and *C. frutescens* revealed the presence of viruses belonging to the families *Bromoviridae*, *Endornaviridae*, *Solemoviridae*, *Potyviridae*, *Partitiviridae*, and *Virgaviridae*.

PMMoV (*Virgaviridae*) was the most abundant virus in the viromes of both *Capsicum* species, represented by 897 contigs and accounting for 40.1% of the viral reads in *C. annuum* and 51.2% in *C. frutescens* (Table 2; Fig. 2). This was followed by cucumber mosaic virus (CMV; *Bromoviridae*), which comprised 1,197 contigs and approximately 20% of the viral reads in each plant library. The virus species *Cucumovirus CMV* and *Polerovirus PEVYV* were detected in both *A. gossypii* and *Capsicum* plants (Table 2; Fig. 2). In the *A. gossypii* library, *Polerovirus PEVYV* dominated the virome, represented by 81 contigs and accounting for 98.9% of the viral reads, whereas CMV comprised 41 contigs (1.1% of the aphid virome reads). In contrast, *PeVYV* represented 6.7% of the viral reads in the *C. annuum* library and 1% in the *C. frutescens* library.

Pepper severe mottle virus (a putative member of the family *Potyviridae*) was detected exclusively in *C. annuum* (13.3% of viral reads) and *C. frutescens* (2.5% of viral reads) (Table 2; Fig. 2). Additionally, members of the *Partitiviridae* family, *Deltapartitivirus unocapsici* and *Deltapartitivirus duocapsici*, as well as *Alphaendornavirus capsici* (*Endornaviridae*), were also identified, although each represented less than 3% of the viral reads (Table 2; Fig. 2).

Phylogenetic results are illustrated in supplementary material (Supplementary figures S1-S6). Sequences reported in this study were submitted to GenBank under the accession numbers PV029270-PV029280.

Diversity indices indicated *A. gossypii* showed the lowest viral diversity (Shannon index $H=0.6$; Simpson index $D=0.8$) compared with *C. frutescens* ($H=1.2$; $D=0.4$) and *C. annuum* ($H=1.2$; $D=0.6$). When comparing the composition of host plant and aphid vector virome, by means of a similarity analysis, specifically, Hutcheson's t-test for diversity, it was found that there are significant differences in species richness and diversity between the viromes of *A. gossypii* and the chili pepper plants, *C. annuum* ($t\text{-value}=4.8$; $p\text{-value}<0.0001$) and *C. frutescens* ($t\text{-value}=5.2$; $p\text{-value}<0.0001$). However, between *C. annuum* and *C. frutescens*, no significant differences were evident ($t\text{-value}=-0.5$; $p\text{-value}>0.05$).

The genomic coverage of each detected virus was estimated (Table 2). However, in the case of pepper severe mottle virus (PepSMoV), the sequences showing the highest homology did not correspond to complete genomes, but to partial coding regions (CDS), in which 99.30% similarity and 20%-32% coverage were found with the 2018 bp sequence of PepSMoV isolated from *Capsicum chinense* from Palmira, Colombia (GenBank Accession No. MK423930.1). Phylogenetic analysis revealed that they cluster within a clade distinct from other PepSMoV sequences previously reported in Colombia and Venezuela (Figure S1). The average genome size obtained, from 35 complete PepSMoV genomes (Table S1), is 9589 bp, which is equivalent to the sequence recovered from the cayenne pepper library (9839 bp). The partial genome recovered for pepper severe mottle virus (PepSMoV) presented an ORF coding for a 3101 aa polyprotein, in which conserved domains for RNA-dependent RNA polymerase RdRp (interval 7571–8278), Helper component proteinase (interval 1118–2440), coat protein (interval 8777–9475) are evident, Protein P3 of potyviral polyprotein (interval 2549–3859), *Potyviridae* polyprotein (interval 4859–5677), Peptidase family C4 (interval 6344–7045), Potyvirus P1 protease (interval 338–1069), DEAD-like helicases superfamily (interval 3944–4396), helicase superfamily c-terminal domain (interval 4490–4786); These predictions were made based on reference genomes and conserved domains of the *Potyviridae* family available in public databases (Fig. 3).

The sequence of viral origin obtained from the cayenne pepper library and identified as bell pepper endornavirus (BPEV; *Alphaendornavirus capsici*, *Endornaviridae*) achieved 99% coverage with respect to the reference genome. It presented an ORF whose product was a polyprotein of 4889 amino acids (aa) with the following

Table 2 Classification and characterization of contigs of viral origin in the analyzed libraries, whose identity with respect to the reference sequences was greater than 90% and whose coverage was greater than 60% in some of the libraries

Virus	NCBI TaxID	Reference genome accession code	Source	Genome type	RNA	Reference genome length bp.	Length bp. (genome coverage) by each library			Features		
							A. <i>gossypii</i>	C. <i>annuum</i>	C. <i>frutescens</i>	ORF	Length nt aa	Protein Name*
Bell pepper endor- navirus (BPEV; <i>Alphaendorna- virus capsici</i> , <i>Endornaviridae</i>)	354,328	NC_039216	RefSeq	Monopartite	1	14,714	0	14,773 (99%)	6869 (46.6%)	1	14,670 4889	Polyprotein
	369,643	NC_037095	RefSeq	Bipartite	1	1563	0	1640 (97%)	538 (34%)	1	1440 479	Putative RNA-dependent RNA polymerase [Pepper cryptic virus 1]
		NC_037096	RefSeq	2	1512	0	1668 (89%)	1668 (89%)	1	1272 423	Putative coat protein	
Pepper cryptic virus 2 (PCV2; <i>Deltapar- titivirus diocapsici</i> , <i>Partitiviridae</i>)	1,050,903	NC_034159	RefSeq	Bipartite	1	1609	0	1600 (99%)	1509 (94%)	1	1002 333	RNA-dependent RNA poly- merase [Pepper cryptic virus 2]
		NC_034167	RefSeq	2	1525	0	1575 (97%)	1510 (98%)	1	1293 430	coat protein [Pepper cryptic virus 2]	
Pepper mild mottle virus (PMMoV; <i>Tobamovirus capsici</i> , <i>Virgaviridae</i>)	12,239	NC_003630	RefSeq	Monopartite	1	6357	0	4960 (78%)	6441 (98%)	1	3354 1117	replication-associated protein
										2	1410 469	RDRP
										3	774 257	movement protein
										4	474 157	coat protein
Pepper vein yel- lows virus (PeVYV; <i>Polemovirus PEVYV</i> , <i>Solemoviridae</i>)	909,827	NC_015050	RefSeq	Monopartite	1	6244	4331 (69.3%)	4273 (68.4%)	4318 (69%)	1	1965 654	Putative P1 protein [Pod pepper vein yellows virus]
										2	948 315	Putative P1-P2 fusion protein [Pod pepper vein yellows virus]
										3	750 249	P0 protein [Pepper vein yellows virus]
										4	621 206	Coat protein [Pepper vein yel- lows virus]
										5	471 156	Movement protein [Pepper vein yellows virus]
<i>Pepperseveremottlevi- rus</i> (PepSMoV; unclassified <i>Polyviridae</i>)	1,796,097	MK423930.1	GenBank	Monopartite	1	9589	0	9839 (20%)	6024 (32%)	1	9306 3101	Polyprotein
Cucumber mosaic virus (CMV; <i>Cucumovirus</i> <i>CMV</i> , <i>Bromoviridae</i>)	12,305	NC_002034	RefSeq	Tripartite	1	3357	0	3373 (98%)	3420 (97%)	1	2973 990	Replication protein 1a
		NC_002035	RefSeq	2	3050	0	3043 (99%)	3052 (99%)	1	2604 867	RNA-directed RNA polymerase 2a	
				3	2216	608 (27.4%)	2238 (96%)	2056 (93%)	2	333 110	Cucumovirus protein 2B	
										1	852 283	Movement protein
										2	654 217	Coat protein [Cucumber mosaic virus]

* Protein names reflect virus nomenclature prior to the 2023 ICTV update, as listed in the consulted databases (ICTV 2023)

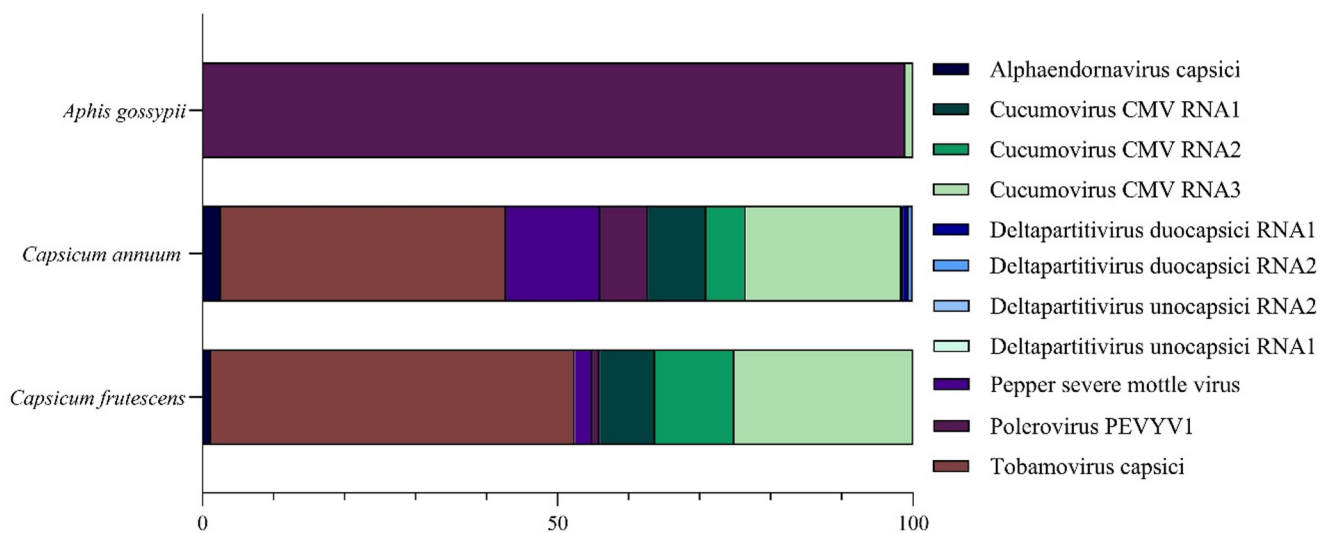


Fig. 2 Relative abundance of viral reads across all libraries

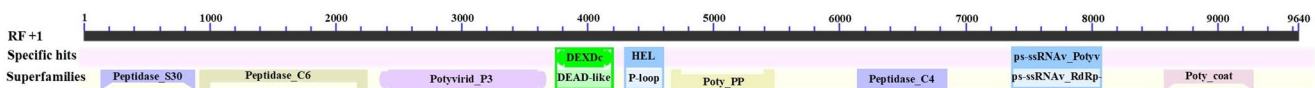


Fig. 3 Predicted conserved domains for the pepper severe mottle virus (PepSMoV), obtained from the cayenne pepper library

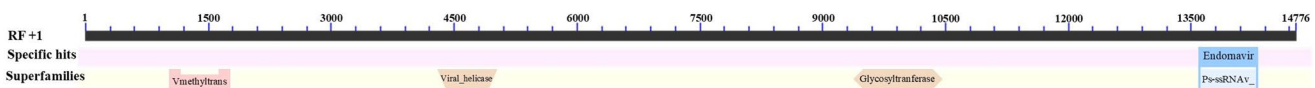


Fig. 4 Conserved domains in bell pepper endornavirus sequence recovered from the cayenne pepper library

domains, RNA-dependent RNA polymerase RdRp (interval 13600–14310), Glycosyltransferase GTB-type super family (interval 9400–10443), Viral methyltransferase (interval 1042–1749), Viral (Superfamily 1) RNA helicase (interval 4300–5010) (Fig. 4). The BPeV sequence identified in this study from the inter-Andean valley of the Cauca River showed a closer phylogenetic relationship with isolates from China and India, rather than with those previously reported from Colombia (Figure S2).

The sequence of partitivirids recovered from the cayenne pepper library, identified as pepper cryptic virus 1 (PCV1; *Deltapartitivirus unocapsici*), presented in its RNA 1, an ORF coding for a protein of 479 aa with RNA-dependent RNA polymerase domain, while RNA 2, contained an ORF coding for a capsid protein (423 aa). Phylogenetic analysis revealed that the Colombian PCV1 strain clusters closely with a South Korean isolate (Figure S4). Similarly, the sequences identified as pepper cryptic virus 2 (PCV2; *Deltapartitivirus duocapsici*), exhibited an RNA1 segment with an ORF encoding 333 aa RNA-dependent RNA polymerase, and an RNA 2 segment with an ORF encoding a 430 aa capsid protein. The Colombian PCV-2 strains recovered from

C. annuum and *C. frutescens* are phylogenetically related to previously reported isolates from the United States, South Korea, and Turkey (Figure S3).

The recovered RNA sequences of cucumber mosaic virus (*Cucumovirus CMV*, *Bromoviridae*) presented in RNA 1 an ORF encoding a 990 aa product corresponding to protein 1a, which is associated with viral genome replication. RNA 2 contained two ORFs: the first encoded a protein of 867 aa with an RNA-dependent RNA polymerase domain, and the second encoded protein 2b, a presumed viral movement protein. RNA 3 also contained two ORFs, the first encoding a movement protein of 283 aa, and the second encoding a 217 aa product corresponding to the capsid protein. Our phylogenetic analysis placed the recovered strains within subgroup IB, which is commonly linked to *Capsicum* infections (Figure S4).

Pepper vein yellows virus (*Polerovirus PEVYV*; *Sol-emoviridae*) found in the cayenne pepper library, presented ORFs coding for P1 protein with peptidase activity (554 aa), P1-P2 fusion protein with RNA-dependent RNA polymerase domain (315 aa), P0 protein associated with viral suppression of RNA silencing (249 aa), capsid protein (206

aa), movement protein (156 aa). The Colombian PCV2 strains recovered from *C. annuum*, *C. frutescens*, and *A. gossypii*, are phylogenetically related to previously reported isolate from China (Figure S5).

The PMMoV (*Virgaviridae*) RNA sequence, recovered from the tabasco chili pepper library, exhibited an ORF 1 encoding for a replication-associated protein (1117 aa) that has methyltransferase and helicase domains, RDRP protein (469 aa) associated with replication activity as it has RNA-dependent RNA polymerase domains, a viral movement protein (257 aa), and a capsid protein (157 aa). Phylogenetic analysis indicates that the Colombian isolate from *C. frutescens* is closely related to strains from Japan and Spain (Figure S6).

Discussion

Previous studies carried out by Morales et al. (2005) and Pardey and García (2011), using the ELISA and RT-PCR techniques, detected pepper deforming mosaic virus (PepDMV) and cucumber mosaic virus in plants showing viral symptoms, particularly internode shortening, leaf curling and yellowing, and concluded that PepDMV is the one with the highest incidence in Valle del Cauca region. Elisa and RT-PCR techniques require prior knowledge of the virus to be detected, so other viruses that could act synergistically and cause the previously described symptomatology are completely ignored (Adams et al. 2009; Boonhama et al. 2014; Wylie et al. 2012). Rivera-Toro et al. (2021), used RT-PCR and PCR techniques to diagnose the presence of pepper severe mottle virus (PepSMo) in *C. chinensis*, with symptoms of viral disease such as leaf deformation and mosaic patterns.

The consistently high Simpson index values across all libraries indicate that the viromes are dominated by a few viral groups, with low evenness among the detected viruses. However, the results also reveal the presence of a diversity of viruses that have been ignored by previous studies and that contribute to the characterization of the symptomatology of viral affection observed in chili pepper plants. These viruses include cryptic viruses (Roossinck 2010), mechanically transmitted symptomatic viruses, and vector-borne symptomatic viruses (Dáder et al. 2017), the latter also found in *A. gossypii*, its presumed vector.

Cryptic viruses

Families such as *Endornaviridae* and *Partitiviridae* are considered cryptic, vertically transmitted, and generally asymptomatic in plants (King et al. 2012; Roossinck 2010).

In our samples, we identified *Alphaendornavirus capsici* (*Endornaviridae*) as well as *Deltapartitivirus unocapsici* and *Deltapartitivirus duocapsici* (*Partitiviridae*). Notably, the *Alphaendornavirus capsici* sequence recovered from the Cauca Valley showed phylogenetic clustering with Asian isolates, contrasting with previously reported Colombian strains, suggesting an independent introduction. The detection of these cryptic viruses across *Capsicum* plants in the region underscores their persistence in commercial crops, although their contribution to host fitness or virus-vector interactions remains unresolved.

Symptomatic vector-transmitted viruses

CMV is a generalist acute virus transmitted by aphids, particularly *A. gossypii* and *M. persicae* (Jacquemond 2012; Kenyon et al. 2014). In line with its known association with solanaceous hosts in warm climates, CMV was one of the dominant viruses in both *C. annuum* and *C. frutescens*.

Pepper vein yellows virus (PeVYV; *Solemoviridae*) was also detected in both *C. annuum* and *C. frutescens*, marking the first report of this virus in Colombia. The symptoms observed in the field (interveinal yellowing, leaf curling, and internode shortening) were consistent with those previously reported for infections associated with PeVYV, although no direct causal relationship was established through single-infection experimental procedures (Alabi et al. 2015; Madroñero et al. 2019; Murakami et al. 2011; Tomassoli et al. 2016; Villanueva et al. 2013; Waweru et al. 2019).

Viral reads were also detected in *A. gossypii*; however, their low abundance indicates that further validation is required. Poleroviruses are transmitted in a circulative, non-propagative manner, the limited number of viral reads may reflect low viral titers in the *Capsicum* plants, resulting in minimal uptake by the aphids, even though *A. gossypii* has been reported as a competent vector for PeVYV (Murakami et al. 2011; Ng and Perry 2004). This underscores the need for future studies addressing vector transmission dynamics within the local agroecosystem.

The aphids of the species *A. gossypii*, characterized in the present study, are recognized vectors of potyviruses and cucumoviruses, which are traditionally associated with viral symptoms in *Capsicum* crops (Brault et al. 2010; Gildow et al. 2008; Pinto et al. 2008a). *Cucumovirus CMV* and *Polerovirus PEVYV* were also detected in the *A. gossypii* virome, however, because of the low number of virus-derived reads and it remains possible that these sequences originated from ingestion of contaminated plant sap rather than from active transmission. Therefore, further studies are required to confirm the role of *A. gossypii* as a CMV and PeVYV vector, and to evaluate whether the viruses reported here are also associated with *M. persicae*.

Pepper severe mottle virus (PepSMoV) is not accepted as a species by the International Committee on Taxonomy of Viruses (ICTV). However, partial coding sequences, available in public databases under this group, allowed its detection in the libraries of *C. annuum* and *C. frutescens*. According to Rivera-Toro et al. (2021), symptoms associated with PepSMoV infection in *C. frutescens* plants are leaf deformation and stunting. Rodríguez-Román et al. (2023), proposed pepper severe mottle virus as belonging to a new species in the family *Potyviridae*, based on the divergence of more than 24% in its nucleotide sequence and more than 18% in the amino acid sequence of the coded product, of the capsid protein of this presumed new virus species with pepper yellow mosaic virus (PepYMV). The symptomatology described by these authors for PepSMoV in *C. annuum* plants is severe mottling and yellowing of leaves. Although PepSMoV was previously reported only in *C. frutescens*, we also detected it in *C. annuum* plants, importantly, we provide new sequence data that could serve as a reference for future taxonomic clarification of this putative species.

In the present study, PepSMoV was not detected in *A. gossypii* but was recovered from the libraries of *C. annuum* and *C. frutescens* with symptoms of virus infection. Considering that viruses of the *Potyviridae* family are transmitted by aphid vectors (Aphididae), it is noteworthy that *M. persicae* was also observed feeding on chili pepper plants in the evaluated crop fields (Duque-Gamboa et al. 2021), suggesting that this species could act as a potential vector facilitating the transmission of the virus to cultivated plants. This hypothesis could be tested through virome analysis of this aphid species, which would help clarify whether active viral transmission is taking place within the agroecosystem context.

In the case of other members of *Potyviridae*, such as pepper mottle virus (PepMoV), it is a virus transmitted by aphids in a non-persistent way. Among its vectors have been reported *A. gossypii*, *Aphis craccivora* Koch and *M. persicae* (Cerkaskas 2004). However, transmission efficiency studies have been carried out only on *M. persicae* finding a transmission efficiency of this virus in *C. annuum* plants from 70% to 100% (Zitter 1975).

Mechanically transmitted viruses

In addition to vector-borne viruses, PMMoV (*Virgaviridae*) was detected, a globally significant pathogen of *Capsicum* crops that is mechanically transmitted through seeds, soil, and direct plant-to-plant contact (García-Luque et al. 1990; Genda et al. 2005; Petrov 2014). When transmission occurs through seeds, the severity of symptoms is observed in young plants, causing losses between 50 and 100% in *C. annuum* crops (Martínez-Ochoa et al. 2003). Since the

sampled plants developed normally until late growth stages, seven months after seedling transplanting, our findings suggest mechanical transmission rather than seedborne infection. Given PMMoV's persistence and its potential for cross-infection with tomato and weed species (Petrov 2014), it is important to evaluate the seed banks used as sources for new plantations and to determine whether other susceptible crops are present at the regional level. It is even relevant to evaluate the presence of this virus in hosts that are not of commercial interest, since this virus has been reported infecting weed plants that are associated with the cultivated area and are considered a source of virus contamination (Kwon et al. 2016).

In conclusion, with the comparison of the viromes of *C. annuum*, *C. frutescens* and *A. gossypii*, it is possible that *A. gossypii* is a vector of symptomatic viruses belonging to families *Bromoviridae* and *Solemoviridae* that affect crop plants, however, further studies are required to explore and validate this hypothesis. On the other hand, these plants were also affected by mechanically transmitted viruses such as PMMoV, which generates a similar symptomatology. This study provides a valuable initial overview of the *Capsicum* virome in the inter-Andean valley of the Cauca River. However, to fully uncover the interactions among viral species and their specific impacts on host plants, it is essential to conduct single-plant analyses to confirm co-infection and assess synergistic effects. PepSMoV was detected in the virome of *Capsicum* spp. plants, but not in the aphid virome, although its taxonomic status remains under discussion, and it has not yet been officially recognized as a species by the ICTV. The genomic information obtained in this study may serve as a valuable reference for future classification efforts. In addition, the presence of asymptomatic persistent viruses such as BPEV, PCV1, and PCV2 has been reported, raising questions about their potential influence on the biological fitness and physiology of the host.

As a working perspective, it is necessary to characterize the infections of these viruses using PCR-based tools, to detect infections and co-infections of these viruses in individual plants. Also, it is necessary to establish the symptomatology of each virus and that resulting from their interaction, since it can be synergistic, potentiating the viral infection.

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Data availability The sequence data generated in this study have been deposited in the GenBank database under accession numbers PV029270 to PV029280.

Declarations

Competing interests We declare that there are no conflicts of interest. This study did not involve any experiments on human participants or animals. All research activities were conducted in accordance with applicable ethical standards and guidelines.

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