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Natural infection of purple passion fruit (*Passiflora edulis* f. edulis) by a novel member of the family *Tymoviridae* in Colombia

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Summary. – Purple passion fruit is one of the most important fruit exports of Colombia, but its productivity is being compromised by the emergence of several viral diseases. High-throughput sequencing (HTS) surveys of viruses in purple passion fruit fields in the province of Antioquia suggested infection by a new member of the family *Tymoviridae.* In this work, we characterize the complete genome sequence of this virus, tentatively named purple passionfruit leaf deformation virus (PpLDV), and evaluate its distribution in Antioquia. PpLDV was assembled at high coverage in four datasets from different regions. The 6.1 kb genome of PpLDV encodes a single polyprotein with domains characteristic of the family *Tymoviridae*, contains a marafibox-like promoter and the 3'-UTR can fold into a tRNA-like secondary structure with a valine anti-codon. Phylogenetic analysis of the polyprotein revealed that PpLDV is a distinct member of the family *Tymoviridae*, more closely related to the genus *Tymovirus* and the unclassified Poinsettia mosaic virus (PnMV). The presence of PpLDV was confirmed by RT-qPCR and RT-PCR in samples from commercial purple passion fruit fields, plantlets and seed sprouts collected in Antioquia using primers designed in this study.

Keywords: high-throughput sequencing; Marafivirus; Passifloraceae; plant virology; RT-qPCR; Tymovirus

Introduction

Purple passion fruit (*Passiflora edulis* f. edulis), a close relative of yellow passion fruit (*P. edulis* f. flavicarpa), has attracted worldwide attention on account of its great potential in the fresh fruit market and food processing industries (Fischer and Rezende, 2008; Melgarejo, 2019). Purple passion fruit is typically consumed fresh, but is also very popular in the preparation of pastries, desserts, yogurts, tea, alcoholic drinks, vinegar, sauces, and sweets (Fischer and Rezende, 2008; Ramaiya *et al*., 2013). In addition to culinary uses, there is also an emerging pharmaceutical interest in the use of *P. edulis* extracts for the treatment of tumors (Kido *et al*., 2020), wounds (Soares *et al*., 2020), depression and anxiety (Alves *et al*., 2020), inflammation (Carmona-Hernandez *et al*., 2019), hypertension, and diabetes (de Faveri *et al*., 2020). Colombia is the top worldwide producer of purple passion fruit with exports totaling about 8700 tons per year mostly to the United Kingdom, Belgium, and the Netherlands (Asohofrucol, 2021). Within the country, the province of Antioquia is the most productive region

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Abbreviations: CABMV = cowpea aphid-borne mosaic virus; CP = coat protein; Ct = cycle threshold; dsRNA = double stranded RNA; GBVA = gulupa bacilliform virus A; Hel = helicase; HTS = high-throughput sequencing; MRFV = Maize rayado fino virus; Mtr = methyl transferase; ORF = open reading frame; PFYMV = passion fruit yellow mosaic virus; Pol = polymerase; PnMV = Poinsettia mosaic virus; PpLDV = purple passionfruit leaf deformation virus; Pro = protease; PVDP = Plant-Virus-Detection-Pipeline; RNAseq = RNA sequencing; RT-PCR = reverse transcription PCR; RT-qPCR = reverse transcription quantitative real-time PCR; SMV = soybean mosaic virus; TYMV = Turnip yellow mosaic virus; UTR = untranslated region

of purple passion fruit with a yield estimated at 22.2 t/ha, which is significantly higher than the national average of 16.3 t/ha (Agronet, 2021). Purple passion fruit has become one of the most important agricultural export articles of Colombia, together with banana, cape gooseberry and avocado, representing an annual income of approximately 31 million USD (Melgarejo, 2019; Asohofrucol, 2021).

Unfortunately, the productivity of purple passion fruit in Colombia started to be seriously compromised by the rapid emergence of different viral diseases that remained uncharacterized until recent years (Jaramillo *et al.*, 2018, 2019; Sepúlveda *et al.*, 2021). To address this problem, a series of high throughput sequencing (HTS) surveys have been performed in the province of Antioquia. These studies have revealed that the most prevalent viruses infecting purple passion fruit in the region are the potyvirus soybean mosaic virus (SMV), the tymovirus passion fruit yellow mosaic virus (PFYMV), the cucumovirus cucumber mosaic virus (CMV) and a new badnavirus tentatively named gulupa bacilliform virus A (GBVA) (tentative binomial name: Badnavirus passiflorae) (Jaramillo *et al.*, 2018, 2019; Sepúlveda *et al.*, 2021). In addition, serological and PCR-based methods suggest the circulation of the potyvirus cowpea aphid-borne mosaic virus (CABMV) in the province of Cundinamarca (Camelo, 2010; Gordillo, 2011). Worldwide, about twenty virus species from the genera *Potyvirus*, *Polerovirus*, *Rhabdovirus*, *Carlavirus*, *Cucumovirus*, *Tobamovirus*, *Tymovirus*, *Nepovirus* and *Begomovirus* are known to infect *P. edulis* (Fisher and Rezende, 2008; Jaramillo *et al.*, 2019; Liberato and Zerbini, 2020); however, this number is expected to increase because of new virus discoveries using HTS. In this work, we provide evidence for the existence of a new virus of the family *Tymoviridae* naturally infecting purple passion fruit stands in the province of Antioquia using HTS, real-time RT-PCR (RT-qPCR), RT-PCR and Sanger sequencing.

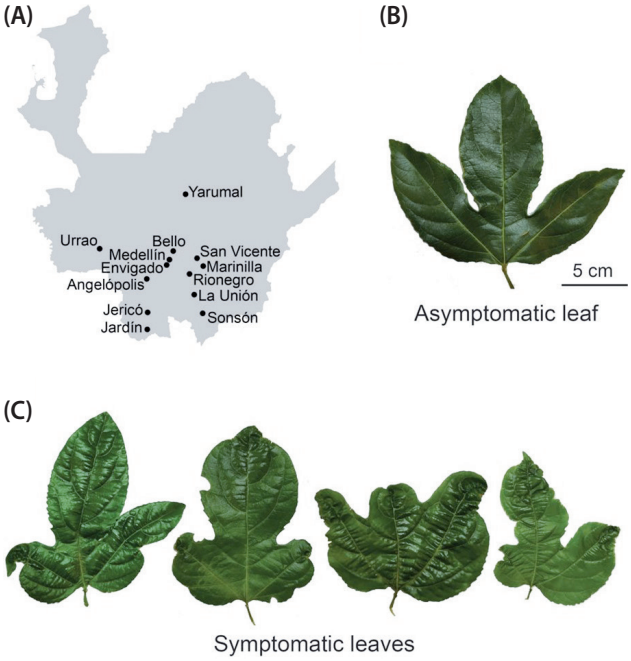

Fig. 1

Collection sites and symptoms of viral infection in purple passion fruit

(A) Samples used in this study were collected in different municipalities from southwest, east, central, and northern Antioquia. **(B)** Asymptomatic leaves exhibited the typical leaf morphology of *P. edulis* f. edulis consisting of three-lobed leaves with serrate margins and a glossy surface. **(C)** Symptomatic leaf samples exhibited extreme deformation characteristic of viral infections.

Materials and Methods

Plant material. The four RNAseq datasets used in this study comprised samples from different tissues, RNA inputs, and regions within the province of Antioquia (Colombia) (Table 1; Fig. 1A). Datasets BPE19 and BPE20 were generated from asymptomatic and symptomatic leaves collected at commercial plots

Table 1. Description of the RNAseq datasets used in this study

Sample name	Tissue	Municipalities	Extraction method	Number of reads	*Tymoviridae*-like sequences (percent identity)	Other viruses in the sample
BPE19	Asymptomatic leaves	Jardín Jericó	Total RNA (Trizol)	12,042,816	2,729 (81.9%)	GBVA
BPE20	Symptomatic leaves	Jardín Jericó	Total RNA (Trizol)	13,373,989	874 (81.8%)	GBVA SMV
BPE24	Seed embryos	Jardín Jericó	Total RNA (GeneJET)	12,385,103	889 (81.7%)	GBVA SMV
BPE8-9	Randomly collected leaves	Sonsón Rionegro San Vicente	dsRNA (Valverde *et al.*, 1990)	15,132,801	10,182 (81.4%)	GBVA PFYMV SMV

in the municipalities of Jardín (5º 35' 50'' N y 75º 50' 50'' W) and Jericó (05°46'59''N, 75°46'59''W) in southwestern Antioquia; dataset BPE8-9 was derived from a bulked sample of randomly collected leaves from commercial purple passion fruit stands in the municipalities of Sonsón (5.7120° N, 75.3098° W), San Vicente (6.3033° N, 75.3179° W) and Rionegro (6.1409° N, 75.4112° W) in eastern Antioquia; the fourth dataset, BPE24, was generated from embryos of seeds collected in Jardín and Jericó. When observed, viral symptoms in adult plants included leafrolls, leaf deformations and rugose mosaics (Fig. 1B,C).

RNA extraction. Prior to extraction, plant tissue was ground into a fine powder using a mortar and a pestle in liquid nitrogen. RNAseq inputs for datasets BPE19 and BPE20 consisted of total RNA extracted from 100 mg of tissue using the Trizol method (Chomczynski and Sacchi, 1987). For the BPE24 dataset, total RNA was purified from 100 mg of seed-embryos using the GeneJET Plant RNA Purification Mini Kit (Thermo Fisher Scientific, USA). Dataset BPE8-9 was generated from double stranded RNA (dsRNA) purified from 5 g of leaf tissue using the procedure described by Valverde and collaborators (1990) with Cellulose fibers (C6288 Medium, Merck, USA) equilibrated in 16.5% STE buffer (100 mM NaCl, 10 mM Tris-Cl, 1 mM EDTA at pH 8.0). Total RNA for quantitative (RT-qPCR) and standard RT-PCR was prepared by the Trizol method and eluted in 40 µl of DEPC-treated water and preserved at -20°C until further use. RNA concentration and purity were determined using the A260/280 ratio measured in a Nanodrop 2000C spectrophotometer (Thermo Fisher Scientific, USA).

RNAseq. The quality and quantity of the RNA inputs for sequencing were assessed with a 2100 Bioanalyzer (Agilent Technologies, USA). cDNA libraries for high-throughput RNA sequencing were synthesized with the TruSeq Stranded Total RNA LT Sample kit (Illumina, USA); ribosomal RNA (rRNA) was removed with the Ribo-Zero Plant kit (Illumina, USA). Sequencing was performed with the Illumina NovaSeq system service provided by Macrogen (South Korea). High-throughput sequencing resulted in datasets of 12,042,816 (BPE19), 13,373,989 (BPE20), 12,385,103 (BPE24), and 15,132,801 (BPE8-9) 101 nt paired-end reads (Table 1).

RT-qPCR and RT-PCR. Synthesis of cDNA as the template for qPCR was carried out in a reaction volume of 20 µl, including 200 U of RevertAid Reverse Transcriptase (Thermo Fisher Scientific, USA), 1X RT buffer, dNTPs at 0.5 mM each, 20 units of RiboLock, 50–100 ng of total RNA, and 20 pmoles of reverse primer Gulupa_tymR_CP_6413 (5'-GATTCAGCGACG GACAACC-3'). Reactions were first incubated for 5 min at 65°C, followed by 1 hour at 42°C, and an inactivation step of 10 min at 70°C. Real-time PCR reactions were carried out in a volume of 14 µl that included 50–100 ng of cDNA, 0.3 µM of primers qGulupa_tymF_CP_6314 (5'-CTCCGCCTAAGCCACCTTG-3') and Gulupa_tymR_CP_6413, and 6.25 µl of Maxima SYBR Green/ROX reagent (Thermo Fisher Scientific, USA). Amplification of 100 bp of target sequences was performed under the following cycling conditions: initial denaturation at 95°C for 10 min, followed by 35 cycles with 15 s at 95°C, and 60 s at 52°C. All reactions were carried out in a Rotor-Gene Q-5plex cycler (Qiagen, Germany), and data analyzed using the Q-Rex Software. Melting curves were acquired on the green channel by measuring the change in fluorescence in the 50–99°C range. Each run included a positive control sample for the new virus; negative controls contained all the elements of the reaction mixture minus the template. The sequence of selected RT-qPCR amplicons was confirmed by Sanger sequencing after purification from a 2% agarose gel using the GeneJET Gel Extraction kit (Thermo Fisher Scientific, USA). Partial genome amplification was performed by RT-PCR using the Verso 1-step RT-qPCR Kit (Thermo Fisher Scientific, USA) with primers Gulupa_tymF_CP (5'-CTCAACCTTCTGTC CACTCC-3') and Gulupa_tymR_CP_6413 targeting the CP region; and Gulupa_tymF_MT (5'-CCTCGTCCCTTTCCTATCCC-3') and Gulupa_tymR_MT (5'-AGAGCGTCATACACCTTTCG-3') targeting the Mtr region. All Primers were designed with PrimerBLAST (Ye *et al*., 2012) using the assembled genome as a reference.

Bioinformatic analyses. Preliminary detection of viral sequences was performed with the Plant Virus Detection Platform, PVDP (Gutiérrez *et al*., 2021). Viral sequences were assembled *de novo* with rnaSPAdes (Bushmanova *et al*., 2019). High-quality contigs from rnaSPAdes exhibiting significant sequence similarity to members of the family *Tymoviridae* were selected with dc-megablast (Altschul *et al*., 1997) using a custom database comprising all available sequences at NCBI (https://www.ncbi.nlm.nih.gov/). Assemblies were inspected manually for potential errors using the Integrative Genomics Viewer software (Robinson *et al*., 2011) after mapping reads with MagicBLAST (Boratyn *et al*., 2019). The open reading frame (ORF) encoding the viral polyprotein and its protein motifs were identified with ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/), and PFAM (El-Gebali *et al*., 2019), respectively. RNA secondary structural motifs were identified with RNAfold (Gruber *et al*., 2008). Phylogenetic analyses were performed in MEGA X with the Neighbor-Joining method and 1000 bootstrap replicates (Kumar *et al*., 2018). The evolutionary distances were computed using the Jones-Taylor-Thornton method (JTT) (Jones *et al*., 1992) for amino acid sequences and the Tamura-Nei method (Tamura and Nei, 1993) for nucleotide sequences. Bioproject read sequences were submitted to the NCBI nucleotide database under accession codes: SRR17201899, SRR17201902, SRR17201903 and SRR17201904. The final curated PpLDV genomes have the accession codes: OL828767 to OL828770.

Results and Discussion

Identification, assembly, and characterization of tymoviridae-like sequences

In addition to viruses known to infect purple passion fruit in Colombia such as SMV, PFYMV, and GBVA, analy-

sis of purple passion fruit datasets from adult plants and planting material using the PVDP platform (Gutiérrez *et al.*, 2021) often detected a large number of sequences with nucleotide identities in the 81.4 to 81.9% range to members of the genera *Tymovirus, Marafivirus*, and *Maculavirus* (Table 1). Data mining of *de novo* assemblies containing tymoviridae-like sequences revealed the presence of contigs of about 6.5 kb in length with very high sequence coverages of 872.0x (BPE19), 359.3x (BPE20), 177.6x (BPE24), and 4,612.8x (BPE8-9) and molecular features typical of members of the family *Tymoviridae* (Fig. 2A). The assembled contigs contained a single open reading frame of 6,137 nt (185-6,322) encoding a polyprotein of 2,405 amino acids with a theoretical molecular weight of 227.7 kDa (Fig. 2A). The predicted polyprotein contains a viral methyltransferase domain (Mtr, Pfam: PF01660, E-value: 2.9e-59) typical of ssRNA viruses (Kong *et al.*, 1999) at amino acid positions 38–319; an endopeptidase domain (Pro, Pfam: PF05381, E-value: 5.5e-18) of the Merops family C21 typical of tymoviruses (Rozanov, *et al.*, 1995) at 750–857; a viral helicase domain (Hel, Pfam: PF01443, E-value: 2.3e-44) (Ahola *et al.*, 2000) at 983–1,171; an RNA-dependent RNA-polymerase domain (Pol, Pfam: PF00978, E-value: 1.1e-13) at 1,440–1743; and a tymovirus coat protein domain (Pfam: PF00983, E-value: 2.9e-30) at 1,864–2044. Genome expression of viruses in the family *Tymoviridae* involves autocatalytic cleavage of the replication polyprotein, which are predicted at positions 854, and 1,224 between the Pro/Hel, and Hel/Pol domains (Fig. 2A, B). A motif with the sequence 5'-CAGGGUGAAU UGCUUC-3', and identical to the marafibox found in maize rayado fino virus (MRFV) and Poinsettia mosaic virus (PnMV), was identified at positions 5,690–5,710 (Fig. 2C). Inspection of untranslated 5'-terminal regions for the formation of secondary RNA structures revealed the potential formation of three stem-loops at positions 48–86, 100-134, and 151–166 with free energies of -10.31, -11.7, and -4.71 kcal/mol, respectively (Fig. 2D). The assembled contigs do not contain a poly(A) tract 3' end, however, the 3'-UTR can fold into a tRNA-like secondary structure of -35.7 kcal/mol at positions 6,376-6,487 with four stem-loops and a 5'-CAC-3' Val anti-codon in agreement with

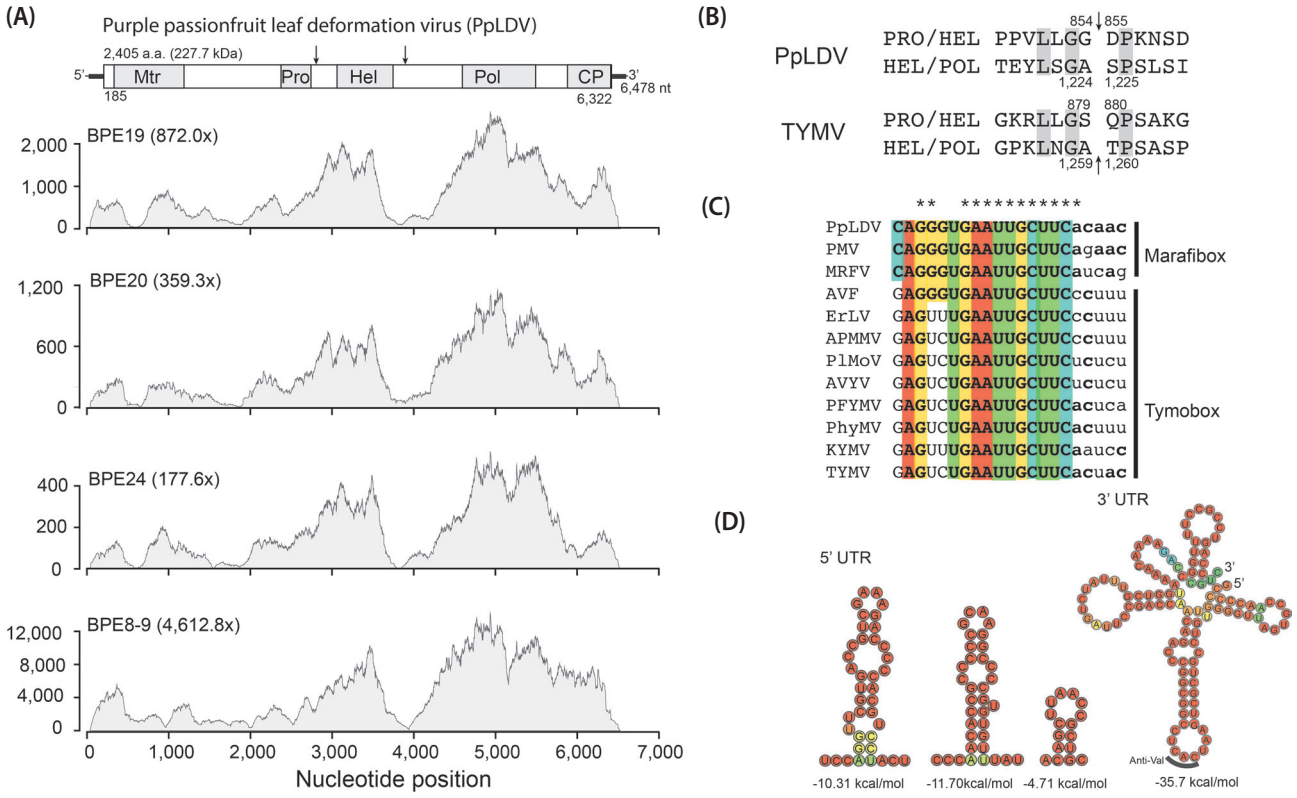

Fig. 2

General description of the purple passionfruit leaf deformation virus (PpLDV) genome

(A) Genome feature of the assembled genomes illustrating the relative position of functional domains and putative protease cleavage sites (arrows). Sequence coverage plots for each assembly are shown at the bottom. **(B)** Predicted protease cleavage sites of the PpLDV polyprotein compared with respect to TYMV. **(C)** Sequence alignment of the marafi- and tymoboxes with the corresponding sequence in PpLDV. **(D)** Secondary structure elements predicted at the 5'- and 3'- untranslated regions of PpLDV.

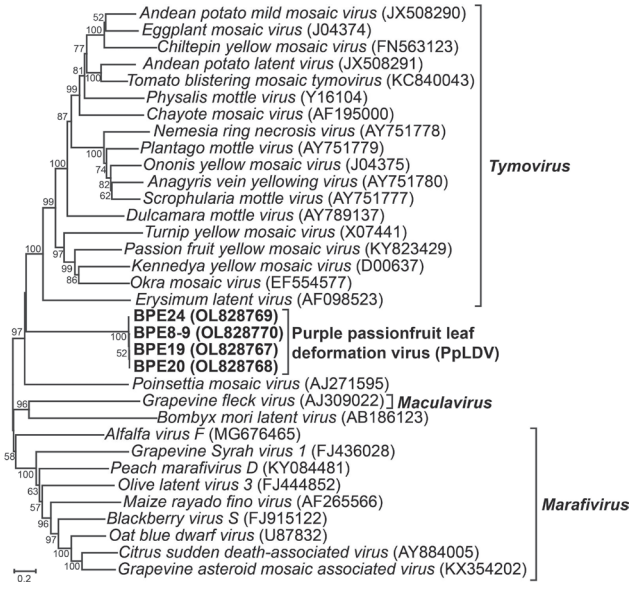

Fig. 3

Relationship of purple passionfruit leaf deformation virus (PpLDV) to other members of the family *Tymoviridae*
The phylogenetic tree obtained using the Mtr-Pro-Hel-Pol region of the polyprotein reveals that PpLDV is a distinct member of the family *Tymoviridae*, basal to the genus *Tymovirus*, and related to Poinsettia mosaic virus (PnMV) yet to be assigned a genus within the family.

the 3'-terminal tRNA-like structures of some members of the family *Tymoviridae* (Rietveld *et al.*, 1982; Stephan *et al.*, 2008) (Fig. 2D).

A comparison of amino acid sequences of functional domains revealed 30.8–64.1% identities with the genera *Marafivirus*, *Maculavirus* and *Tymovirus* in the family *Tymoviridae* (Table 2). The most similar segments corresponded to the Mtr, Hel, and Pol domains with identities in the 46.8 to 64.1% range. The Pro and CP regions were the most variable regions with amino acids identities in the 26.9–43.3% range and nucleotides identities in the 39.4–50.4% range (Table 2). A phylogenetic analysis based on the amino acid sequences corresponding to the Mtr-Pro-Hel-Pol segment of approved members of the family *Tymoviridae* revealed that all assembled sequences formed a well-defined cluster with 100% bootstrap and basal to known members of the genus *Tymovirus* and closely related to PnMV, which has yet to be assigned to a genus (Fig. 3). This result suggests that the identified virus probably represents a new tymoviridae species, for which we suggest the name purple passionfruit leaf deformation virus (PpLDV).

Currently, the family *Tymoviridae* comprises the genera *Tymovirus* (28 species), *Marafivirus* (11 species), and *Maculavirus* (one species), in addition to Bombyx mori latent virus (BmLV) and Poinsettia mosaic virus

Table 2. Comparison of the PpLDV amino acid sequences of functional domains with equivalent regions from different members of the family *Tymoviridae*

Virus species	MTr	Pro	Hel	Pol	CP
Tymovirus					
Andean potato mild mosaic virus (APMMV)	54.0 (55.5)	39.8 (42.3)	50.6 (53.0)	63.9 (60.5)	34.4 (48.7)
Physalis mottle virus (PhyMV)	56.3 (56.4)	35.5 (46.6)	47.2 (51.9)	58.9 (59.0)	31.8 (44.1)
Plantago mottle virus (PlMoV)	59.0 (56.7)	38.7 (39.8)	53.2 (53.7)	60.7 (58.8)	35.0 (48.7)
Anagyris vein yellowing virus (AVYV)	57.0 (58.3)	33.3 (39.8)	53.6 (54.9)	60.8 (59.1)	38.9 (49.6)
Turnip yellow mosaic virus (TYMV)	59.3 (56.8)	37.6 (45.9)	49.6 (53.7)	60.3 (60.0)	30.8 (45.5)
Passion fruit yellow mosaic virus (PFYMV)	59.2 (56.7)	34.4 (46.2)	54.0 (55.3)	60.0 (59.5)	36.9 (48.5)
Kennedya yellow mosaic virus (KYMV)	63.8 (59.9)	36.6 (41.6)	49.4 (51.5)	59.6 (60.8)	35.7 (50.4)
Erysimum latent virus (ErLV)	55.4 (55.2)	26.9 (44.4)	49.4 (54.6)	58.6 (58.1)	36.5 (47.3)
Maculavirus					
Grapevine fleck virus (GFkV)	50.0 (53.8)	38.2 (46.8)	53.6 (56.6)	57.9 (58.9)	31.9 (46.4)
Marafivirus					
Alfalfa virus F (AVF)	52.2 (56.0)	37.6 (46.2)	55.3 (57.0)	62.9 (61.7)	39.8 (49.2)
Maize rayado fino virus (MRFV)	53.7 (56.7)	43.3 (48.5)	54.0 (54.3)	61.5 (57.7)	38.7 (49.1)
Unclassified					
Poinsettia mosaic virus (PMV)	51.8 (54.5)	41.9 (46.2)	55.3 (55.2)	64.1 (61.1)	36.9 (49.2)
Bombyx mori latent virus (BmMLV)	47.8 (51.5)	30.1 (39.4)	46.8 (51.2)	56.8 (57.8)	33.1 (44.3)

Sequence identities (%) at the nucleotide level are shown in parentheses.

(PnMV), which has not been assigned to a genus (ICTV Master Species List #3, 6 March 2021; https://talk.ictvonline.org). Viruses in this family have isometric virions non-enveloped, about 30 nm in diameter with a single-stranded RNA genome of positive sense of about 6.0–7.5 kb, high cytosine content (32–50%), a 5'end m7G cap and most of them have a tRNA-like at the 3'-end (King *et al.*, 2012). At the molecular level, a genus within the family can be distinguished based on the number of ORFs, the presence of characteristic regulatory sequences, and molecular features of the 3'UTR: the genus *Tymovirus* contains three ORFs, a tymobox, and a tRNA-like structure at the 3'-end; *Marafivirus* encode a single ORF, contain a marafibox, and have 3'-terminal poly(A) tail, while *Maculavirus* have four ORFs and a poly(A) tail. PpLDV has a C content of 32.0% and shares several features typical of the genus *Marafivirus*: it lacks an ORF encoding for a movement protein (MP), encodes a single polyprotein, and contains a sgRNA promoter identical to the marafibox. However, the genome of marafiviruses, and also of maculaviruses and PnMV, is polyadenylated at the 3' terminus; this contrasts with the $tRNA^{val}$-like motif found in PpLDV, which is most typical of tymoviruses. PpLDV is also phylogenetically more closely related to members of the genus *Tymovirus* than all other known genera (Fig. 3; Table 2). PpLDV is clearly a new species within the family *Tymoviridae* and probably represents a member of a new genus; future work should clarify the taxonomic status of this virus.

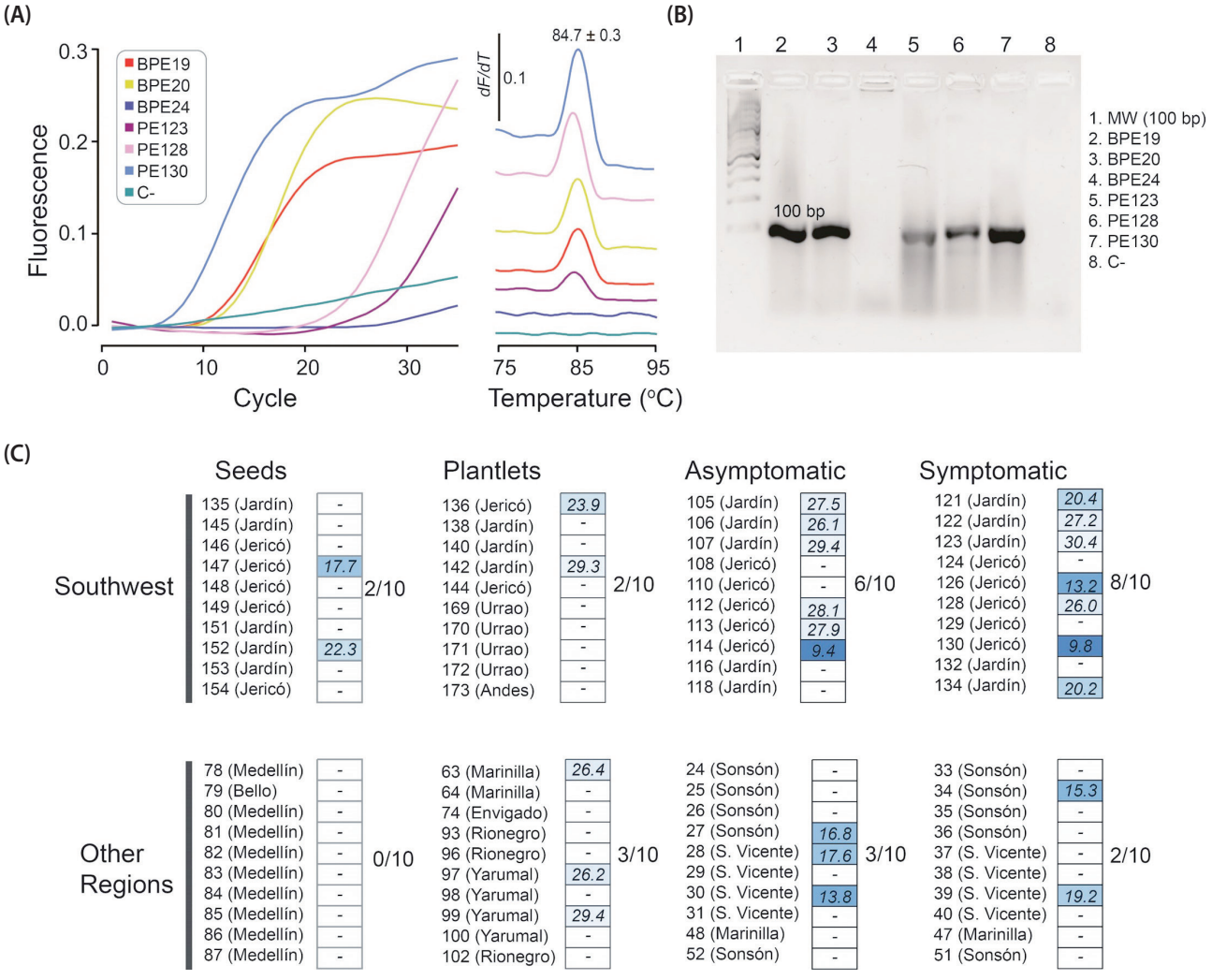

Fig. 4

Detection of PpLDV by RT-qPCR in purple passion fruit samples from different regions in Antioquia

(A) Amplification (left) and melting profiles (right) of amplicons using PpLDV-specific primers designed in this work. **(B)** Agarose gel electrophoresis showing bands with expected size. **(C)** Prevalence of PpLDV in seeds, plantlets, and asymptomatic and symptomatic adult plants from different regions in Antioquia.

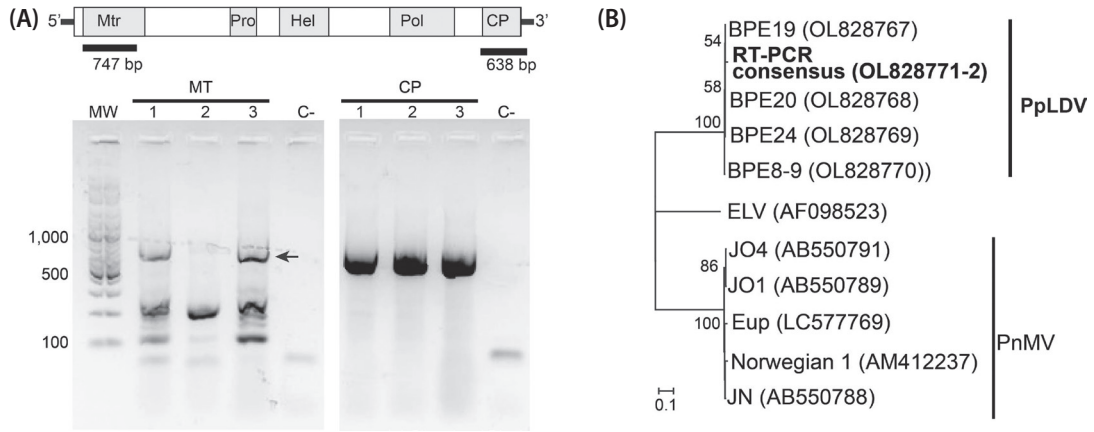

Fig. 5

Detection of PpLDV by standard RT-PCR

(A) Agarose gel electrophoresis of amplification products using specific primers targeting the Mtr and CP regions of the PpLDV polyprotein. Bands of the expected molecular size were observed in each case. For the Mtr amplifications products, the arrow indicates the bands purified for Sanger sequencing. **(B)** Neighbor-joining tree of the consensus Sanger sequence comprising the Mtr and CP amplicons confirms the identity of the amplified virus as PpLDV.

RT-qPCR and RT-PCR

The presence of PpLDV was confirmed in a new set of samples from commercial purple passion fruit fields, plantlets and seeds sprouts collected in the municipalities of Jardín, Jericó, Urrao, Angelópolis, Sonsón, San Vicente Ferrer, Marinilla, Yarumal, Rionegro, Bello, Envigado, and Medellín (Fig. 1A). Detection was performed using RT-qPCR with specific primers targeting the 6314–6413 CP region. Amplicons exhibited a melting point of 84.7 ± 0.3°C (Fig. 4A), had the expected size of 100 bp (Fig. 4B) and were confirmed by Sanger sequencing (data not shown). In seed sprouts, PpLDV was detected in samples from southwest Antioquia collected at the municipalities of Jericó and Jardín. In plantlets, this virus was detected in one sample from Marinilla (east), two from Yarumal (north), and one from Jardín, and Jericó in the southwest (Fig. 4C). PpLDV was most prevalent in adult plants, being detected in six asymptomatic samples from Jardín and Jericó, two from San Vicente Ferrer and one from Sonsón. In symptomatic plants, the virus was detected in eight samples from Jardín and Jericó, and one sample from Sonsón and San Vicente Ferrer (Fig. 4C). Viral loads ranged from 9.4 to 28.1 and were higher in samples collected from Jericó with Ct values of 9.4 in one asymptomatic sample, and 9.8 and 13.2 in symptomatic samples (Fig. 4C). In addition to RT-qPCR, the presence of PpLDV was confirmed by RT-PCR amplification of regions within the MTr (747 bp) and CP (638 bp) domains of PpLDV from three RT-qPCR positive samples (Fig. 5A). Amplicons shared 97.1–100% nucleotide sequence identities with the assembled PpLDV genomes and phylogenetic analysis of the Sanger consensus formed a well-defined clade for PpLDV with 100% bootstrap and clearly separated from its closest relatives Erysimum latent virus (ELV) and PnMV (Fig. 5B).

This work strongly suggests the existence of a new virus member of the family *Tymoviridae* that naturally infects purple passion fruit in Antioquia (Colombia), and adds yet another species to approximately twenty viruses known to infect this crop across the world (Fisher and Rezende, 2008; Jaramillo *et al*., 2019; Liberato and Zerbini, 2020). The virome of purple passion fruit and related crops remains largely unexplored as evidenced by the recent reports of the begomoviruses (*Geminivirdae*) passion fruit chlorotic mottle virus (PCMoV) and Melochia yellow mosaic virus (MelYMV) discovered in Mato Grosso do Sul in Brazil (Fontenele *et al*., 2018; Spadotti *et al*., 2019); cotton leaf curl Multan virus (CLCuMuV) and its cotton leaf curl Multan betasatellite (CLCuMuB) from Yunnan province in China (Tang *et al*., 2020); and passion fruit leaf distortion virus (PLDV) discovered in yellow passion fruit in Valle del Cauca in Colombia (Vaca-Vaca *et al*., 2017). With respect to RNA viruses, recent reports include cucurbit aphid-borne yellows virus (CABYV) (*Polerovirus*, *Luteoviridae*) reported in mixed infection with CABMV in yellow passion fruit in Bahia (Brazil) (Vidal *et al*., 2018), and the potyviruses Passiflora edulis symptomless virus (PeSV) (*Roymovirus*) from Israel (Jover-Gil *et al*., 2018) and Passiflora virus Y (PaVY) from Guangdong province in China (Chen *et al*., 2021). The information presented here will be of value for future disease management programs and the production of certified planting material of purple

passion fruit in Colombia and other *Passiflora*-growing countries. Future work should address the distribution of this virus in different regions in the country, means of transmission and cross infectivity in related crops such as yellow passion fruit (*Passiflora edulis* f. flavicarpa) and sweet passion fruit (*Passiflora ligularis*).

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