proceedings

MDPI

Proceedings

Picorna-Like Virus Discovered in Wild Lime Psyllid *Leuronota fagarae* Burckhardt (Hemiptera: Psylloidea) †

Douglas S. Stuehler[1,2*], Wayne B. Hunter[2*], Yisel Carrillo-Tarazona[3], Hector Espitia[3], Tracey Bell[4], Hannah R. Mann[5], Sasha-Kay V. Clarke[6], Joseph M. Cicero[7], Jawwad A. Qureshi[8] and Liliana M. Cano[3] *

1. *Douglas S. Stuehler, ORISE participant, DOE/USDA, ARS, Fort Pierce, FL 34945, USA. Email: douglas.stuehler@gmail.com
2. *Wayne B. Hunter, USDA, ARS, 2001 South Rock Road, 2001. South Rock Road, Fort Pierce, FL 34945, USA. Email: wayne.hunter@usda.gov
3. Yisel Carrillo-Tarazona. University of Florida, IFAS, Department of Plant Pathology, Indian River Research and Education Center, 2199 South Rock Road, Fort Pierce, FL 34945, USA. Email: yiselcarrillotar@ufl.edu
3. Hector Espitia, University of Florida, IFAS, Department of Plant Pathology, Indian River Research and Education Center, 2199 South Rock Road, Fort Pierce, FL 34945, USA. Email: hespitianavarro@ufl.edu
4. Tracey Bell, Dept. Cell & Mol. Biol., Florida State University, 319 Stadium Drive, Tallahassee, FL 32306.Email: tnb18@my.fsu.edu
5. Hannah R. Mann, HRM, Ft. Pierce, FL, 34949, USA. Email: HannahRMann@gmail.com
6. Sasha-Kay V. Clarke, University of the West Indies, Kingston, Jamaica, Email: sashakayclarke@yahoo.com
7. Joseph M. Cicero, University of Arizona, School of Plant Sciences, Tuscon, AZ. Email: jmc6@email.arizona.edu
8. Jawwad Qureshi, University of Florida, Southwest Florida Research and Education Center (SWFREC), 2685 SR 29 North Immokalee, FL 34142. USA. Email: jawwadq@ufl.edu
3.*Liliana M. Cano, University of Florida, IFAS, Department of Plant Pathology, Indian River Research and Education Center, 2199 South Rock Road, Fort Pierce, FL 34945, USA. Email: lmcano@ufl.edu

† Presented at the 1st International Electronic Conference on Entomology (IECE 2021), 1–15 July 2021; Available online: https://iece.sciforum.net/.

Citation: Stuehler, D.S., Hunter, W.B., Carrillo-Tarazona, Y., Espitia, H., Bell, T., Mann, H.R., Clarke, S.V., Cicero, J.M., Qureshi, J.M., Cano. L. Picorna-like Virus Discovered in Wild Lime Psyllid Leuronota fagarae Burckhardt (Hemiptera: Psylloidea), in Proceedings of the 1st International Electronic Conference on Entomology, 1–15 July 2021, MDPI: Basel, Switzerland, doi:10.3390/IECE-10365

Published: 30 June 2021

Abstract: As of March 2021, the Family: *Picornaviridae* contained 158 species grouped into 68 different genera. We report the identification of a new *Picornaviridae*-like viral specie isolated from the Wild Lime Psyllid (WLP), *Leuronota fagarae*. Extraction and sequencing of nucleic acid from WLP adult salivary glands identified a 5554 nt sequence with 52.75% identity to *Diaphorina citri* (Asian Citrus Psyllid) picorna-like virus polyprotein and 59.61% identity to the *Bemisia tabaci* (Silverleaf Whitefly) picorna-like virus polyprotein, NCBI BLASTx and BLASTp analysis. Sequence comparisons of amino acids and nucleotides showed consistent similarity and motifs consistent with picorna-like virus polyproteins across 8 known species, with significant E-values of 7e-116 or less. Picornavirus genome polyproteins are around 2100-2400 aa in length, being cleaved into multiple active peptides to allow for viral replication. Phylogenetic comparisons using amino acid and nucleic acid polyprotein sequences showed a diverse radiant group of insect hosts. The discovery of a novel picorna-like virus in WLP whose niche overlaps with the Asian citrus psyllid in the state of Florida, USA, and which is strongly related to the *D. citri* picorna-like virus, provides an opportunity to examine virus host specificity, and modes of transmission between these two psyllid species. Ultimately, research will examine the potential to use these viral pathogens for the management of *D. citri* populations to reduce the transmission of *Candidatus* Liberibacter asiaticus, the bacterial pathogen of citrus trees causing Huanglongbing.

Keywords: Huanglongbing; *Picornaviridae; Diaphorina citri, Iflaviridae;* polyprotein, RdRp, SNPs

1. Introduction

Insects are known to be the largest and most diverse taxonomic class of animals in the world, with over one million classified species [1]. This extensive evolutionary divergence

allows insects to serve as hosts for a wide variety of viral species such as: *Ascoviridae*, *Baculoviridae*, *Parvoviridae*, *Iflaviridae*, *Togaviridae*, and *Rhabdoviridae* [2]. As compared to other viral species discovered in vertebrates, prokaryotes, and plants, the number of viral

species identified in insects is relatively low, considering the vast abundance of insect hosts [3]. Previously, the majority of viral species were identified in insects due to the pathogenetic phenotypes displayed by the insect hosts, however, next generation sequencing technology has allowed for the identification of genetically diverse viruses whose genomes had not previously been sequenced [3]. The wild lime psyllid, *Leuronota fagarae* Burckhardt (Hemiptera: Psylloidea), is an invasive insect whose origins trace to South America. The first report of *L. Fagarae* in the US was in southern Florida, as they were discovered on a citrus relative *Zanthoxylum fagara* (L.) Sarg. (Sapindales: Rutaceae) [4]. Genetic material was collected from a colony of *L. fagarae* [4] maintained at the University of Florida, research station, Fort Pierce, FL [Qureshi lab 2016; Russell et al.2014.

The large virus family, *Picornaviridae*, consists of nonenveloped, positive-sense, single stranded RNA viruses, with a ~30nm icosahedral capsid [5]. Other families closely related to *Picornaviridae*, include *Dicistroviridae*, *Iflaviridae*, *Marnaviridae*, and *Secoviridae*; all are in the Order *Picornavirales* [6]. Viruses in *Picornavirales* have one of the widest host ranges, that includes invertebrates and vertebrates [7]. The RNA viruses in *Picornavirales* contain a single polyprotein that undergoes post-translational modifications. The polyprotein transcript consists of conserved genetic sequences such as an RNA-dependent RNA-polymerase, RNA Helicase, Nudix Hydrolase, and capsid. The *L. fagarae* picorna-like virus-FL isolate (LfPLV) draft genome was identified through Next Generation Sequencing and CAP3 assembly showing evidence for known genes associated with *Picornavirales.*

2. Materials and Methods

2.1. Insect Collection.

Colonies of *L. fagarae* were maintained at the University of Florida, research center, Fort Pierce, FL, U.S.A. on citrus relative *Zanthoxylum fagara* (L.) Sarg (Sapindales: Rutaceae) [Qureshi lab, 2016].

2.2. Tissue Collection, RNA Extraction, cDNA Preparation, Library Sequencing

Salivary glands were dissected from 900 live adult psyllids, who were immediately placed into TRIzol™-LS Reagent (Invitrogen™ #10296028, 200mL), as described in "A stationary tweezer platform for high throughput dissections of minute arthropods and extirpation of their minute organs" [8].

2.3. Bioinformatic Analysis

A metatranscriptomic approach was employed to characterize viral sequences within the TRINITY assembly ortholog batch. Nine contiguous viral sequences were identified by mapping orthologs of interest to a known viral genome (ALJ52073.1). The nine contigs were assembled using CAP3 assembler in Unipro UGENE bioinformatics suite (v38) to yield a 9299 bp sequence [9]. Open reading frames were predicted in each contig using ExPASy 'Translate' [10] before assembly to verify contig completeness and significant coding regions. The resulting translations were then subjected to both BLASTp and tBLASTn analysis in the National Center for Biotechnology Information server [11]. BLASTp analysis of an 1852 amino acid sequence revealed the most significant alignment to polyprotein [*Diaphorina citri picorna-like virus*] [12], with an E-value of 0.0, 99% query cover, and 52.75% identity. The alignment with the highest similarity was the viral polyprotein sequenced from *Bemisia tabaci* (AKC57283.1) with 59.61% identity, 21% query cover, and an E-value of 1e-160, [13]. The tBLASTn analysis of the predicted 1852 AA protein returned the most significant alignment to *Diaphorina citri picorna-like virus* isolate BR1 polyprotein gene, complete coding sequence (KT698837.1) with an E-value of 0.0, 99%

query cover, and 51.02% identity. The top *Diaphorina cirtri* picorna-like virus (DcPLV) alignment from the tBLASTn analysis was converted from a nucleotide to amino acid sequence using ExPASy 'Translate' [10]. The RdRp gene coding region was extracted from the DcPLV polyprotein amino acid sequence on the NCBI website (ALJ52073.1), and compared to the ExPASy translation of the DcPLV complete nucleotide genome (KT698837.1). This comparison allowed for the extraction of the nucleotide sequence for the RdRp gene of the significant DcPLV sequence. An alignment was performed using ClustalW in the UGENE bioinformatics suite [9] between the extracted DcPLV RdRp nucleotide sequence, and the CAP3 assembled genome of the newly identified LfPLV. The alignment revealed a 1404 nt sequence in the LfPLV draft genome that was 65% similar to the DcPLV RdRp nucleotide sequence. The same gene identification process used to identify the RdRp CDS was performed to identify RNA helicase, Nudix Hydrolase, capsid, and the two rhv-like amino acid sequences. Figure 1 shows the location of the six identified genes within the LfPLV genome. After obtaining the RNA-dependent polymerase nucleotide sequence from the LfPLV genome, it was translated into amino acid using the ExPASy 'Translate' server (https://web.expasy.org/translate/), and aligned by ClustalW in UGENE (http://ugene.net) to twenty other RdRp and RdDp sequences [Figure 3] obtained from the top matches of the BLASTp alignment, combined with the metagenomic virus identification reported by Nouri et al, [12]. For phylogenetic analysis, the RNA-dependent polymerase sequence was used for nucleotide and amino acid comparisons, as it is the most conserved sequence within the polyprotein CDS. The least significant E-value recorded of the aligned sequences from the BLASTp analysis was the *Varroa destructor virus 1* sequence with an E-value of 2e-106. Exactly 41 amino acid residues were trimmed from the 3' end of the LfPLV RdRp amino acid sequence, and 159 residues from the 5' end, to obtain an even length alignment of 283 residues before construction of the phylogenetic tree. The phylogenetic tree, [Figure 2] used the Neighbor Joining algorithm in MEGAX: Molecular Evolutionary Genetics Analysis version 11.0.4 [14]. A table with all accession numbers and abbreviations is in the Supplementary Material file (Table S1).

3. Results

Individual alignments were conducted by ClustalX in the Unipro UGENE bioinformatics suite to calculate percent similarity between the identified coding regions depicted in Figure 1 of the LfPLV genome and the DcPLV genome [11]. The similarity calculated between RdRp coding regions was 65% over 1404 nt, *rhv* similarity was 59% over 420 nt and 61% over 564 nt, capsid similarity was 55% over 446 nt, RNA helicase was 67% similar over 369 nt, and Nudix Hydrolase was 53% similar over 249 nt. These alignments also served as verifications for our identified coding regions as the aligned portion of the DcPLV sequence was translated to amino acid via MEGA-X, and compared to the protein sequence recorded in NCBI. All protein sequences were 100% identical, thus confirming we had identified the correct coding regions within our assembly. Phylogenetic comparisons in MEGA-X, with a Neighbor Joining statistical method, and Poisson model, revealed the viral RdRp sequence isolated from WLP salivary glands was closely related to the virus families *Iflaviridae* and *Picornaviridae*, in the order *Picornavirales* [Figure 2]. Iflaviruses are members of a rather new family called *Iflaviridae*, where all members possess a single-stranded, positive-sense RNA genome ranging from 8.5 to 10 kb in length [15]. The genome CDS produces a single polyprotein of roughly 3,000 amino acids that is processed post translation to produce a helicase, a protease (e.g. Nudix Hydrolase), an RdRp, and four structural proteins [15]. All classified iflaviruses are known to exclusively infect insects over a vast range of hosts belonging to the Orders: Hemiptera, Lepidoptera, and Hymenoptera [16]. However, our genome assembly only identified three structural protein coding regions, allowing us to believe the LfPLV is not an Iflavirus.

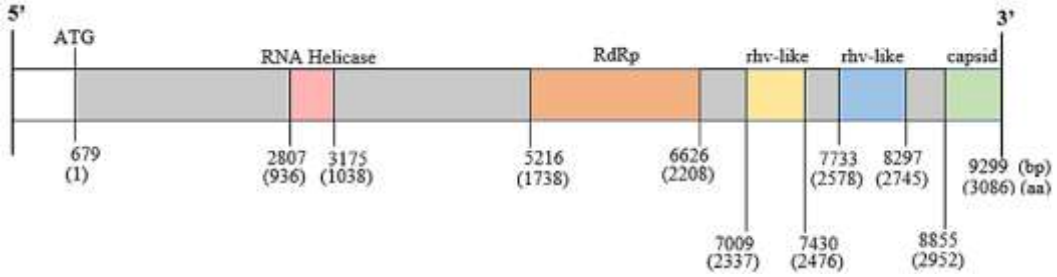

Figure 1. Genome map of draft genome of LfPLV. Identified proteins with identified coding regions from ClustalW alignments to known *D. citri* coding regions shown. Numbers in parentheses are amino acids.

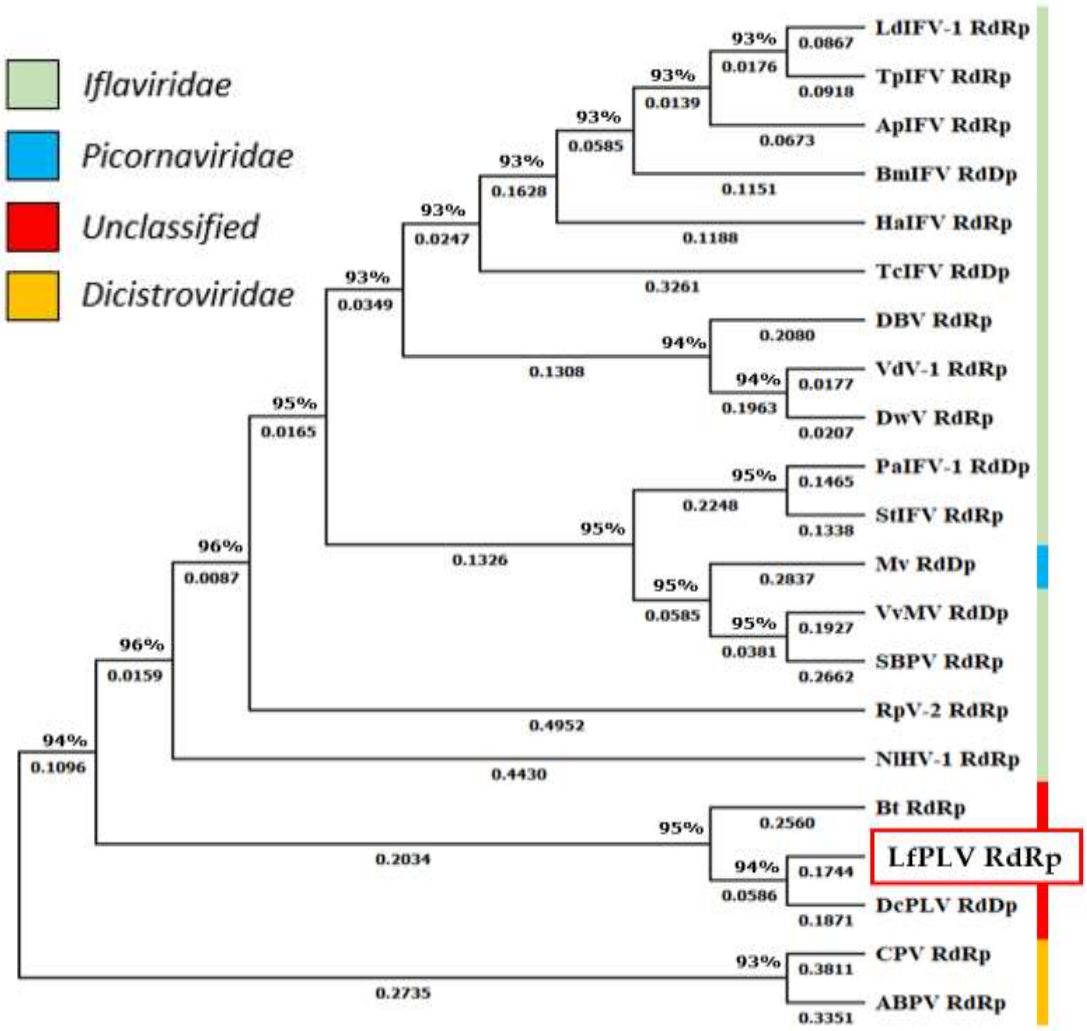

Figure 2. Phylogenetic analysis of Picornavirales amino acid sequences. LfPLV RNA-dependent polymerase sequence forms a clade with two closely related BtPLV RdRp and DcPLV RdRp. Analyses were unrooted. **Note:** See Table S1 for accession numbers and full names.

Two *Dicistroviridae* sequences were included in our phylogenetic analyses for outgrouping as this family exists in the Order, *Picornavirales*, but was not found in any significant BLASTp or tBlastn matches of the LfPLV RdRp sequence. Two Iflaviridae groups emerged during phylogenic analysis, separated by Mornavirus, therefore a denser analysis must be conducted for further classification of the LfPLV taxonomy. An amino acid alignment between DcPLV and LfPLV RNA-dependent polymerase sequences with BLASTp revealed that the two have 65% amino acid similarity promoting our belief that this is the discovery of a new virus species [Figure S2]. All other RdRp sequences displayed in Figure 2 had a 61% or lower similarity to the LfPLV RNA-dependent RNA polymerase sequence. The NJ phylogenic comparison was made with the four closest related RdRp nucleotide sequences of the identified LfPLV RdRp sequence, all sequences were trimmed to 1167 base pairs [Figure S3].

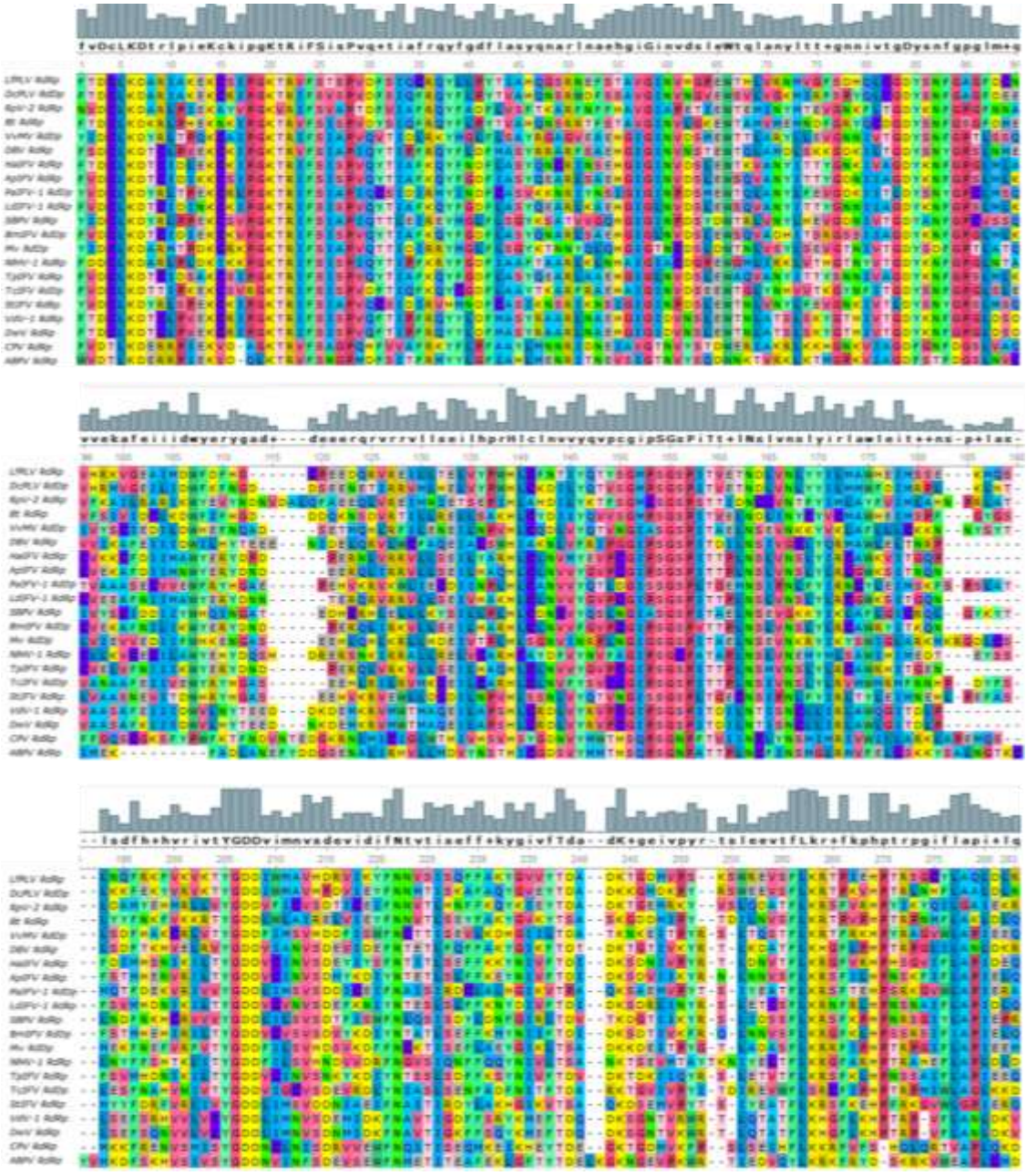

Figure 3. The alignment above was conducted in UGENE by the ClustalW algorithm (v 2.1) and BLOSUM matrix. All sequences were trimmed to the same length before distance calculations by the NJ tree

building method in MEGA-X. **Note:** The LfPLV RdRp protein sequence is placed at the top of the alignment.

4. Discussion

We report on the discovery of a new picorna-like virus, named *Leuronota fagarae* picorna-like virus-Florida isolate, LfPLV-FL. The virus was isolated from salivary glands dissected from the wild lime psyllid, *L. fagarae*, (Hemiptera: Psylloidea). Results from the metagenomic analysis provide strong evidence that LfPLV has close homology to another reported psyllid virus, *Diaphorina citri Picorna-like virus*, [12]. The Asian citrus psyllid, *Diaphorina citri* Kuwayama, (Hemiptera, Psylloidea) is the most destructive insect threatening global citrus production [17]. *Diaphorina citri* is the vector of *Candidatus* Liberibacter asiaticus, the well-known agent of Huanglongbing (HLB) [12]. Huanglongbing is the single most devastating disease of citrus trees, existing as a threat to world citrus production [18]. Currently, there is no cure for HLB, therefore disease control methods rely on various approaches such as psyllid control through biological and chemical-based strategies. The discovery of a picorna-like virus in *L. fagarae* with a close taxonomic relationship to DcPLV provides another potential biocontrol agent for psyllid pests. The picorna-like virus identified in collected *L. fagarae* populations in Florida suggests a high viral persistence in the psyllid host. Insect infecting viruses are gaining attention as expression systems for RNAi to control pests [19, 20].

5. Conclusion

In summary, the analysis of genetic material from *Leuronota fagarae* Florida isolates led to the identification of a psyllid-infecting picorna-like virus. The virus was identified using next generation sequncing methods and bioinformatic analysis. This initial characterization provides a new virus member, in *Picronavirales*. Future examination will focus on modes of transmission and insect host range. The interest is to identify viruses that infect psyllids, which may have use as expression vectors for RNAi biopesticides to control psyllid vectors. Bioinformatic analyses and *in vivo* studies will provide more information for the final classification of LfPLV, picorna-like virus taxonomy. Finally, the discovery of a greater number of viruses that infect psyllids provides a resource that can be used for controlling the Asian citrus psyllid, *D. citri*, and other psyllid vectors of economically important pathogens such as HLB in citrus, and Zebra Chip in potato and tomato.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, **Table S1.** Accession numbers for phylogeny tree comparing LfPLV RdRp to other arthropods, **Figure S1.** Predicted protein translation of LfPLV RdRp coding region, **Figure S2**. Predicted RdRp protein sequence alignment between DcPLV and LfPLV, (NCBI, BLASTp), **Figure S3**. NJ phylogenetic comparison between LfPLV RdRp nucleotide sequence and four of the closest related sequences. **Table S2.** Accession numbers for phylogeny tree comparing LfPLV RdRp nucleotide sequence to four of the closest related sequences.

Author Contributions: Conceptualization, W.B.H.; methodology, W.B.H., L.M.C.., T.B., H.R.M, YCT, HE, J.M.C,; software, L.M.C., D.S.S., S.V.C.; validation, W.B.H., D.S.S.; formal analysis, D.S.S., W.B.H., L.M.C.; investigation, W.B.H., D.S.S., J.A.Q., L.C.; resources, L.M.C., J.A.Q., W.B.H.; data curation, L.M.C., W.B.H., S.V.C., D.S.S.; writing—original draft preparation, D.S.S., W.B.H..; writing—review and editing, W.B.H., J.A.Q., L.M.C., S.V.C.; supervision, W.B.H., J.A.Q., L.M.C.; project administration, W.B.H., J.A.Q., L.M.C.; funding acquisition, W.B.H., L.M.C., S.V.C., J.A.Q. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: All sequence data available at AgriVectors https://www.apsnet.org/meetings/annual/meetingarchives/planthealth2019/Documents/Abstracts/aps2019ab171.htm [21], or www.citrusgreening.org, or contact Wayne.hunter@usda.gov

Acknowledgments: We thank Maria Gonzalez, Biological Science Technician, ARS, Dr. Surya Saha, Bioinformatics Advisor; Dr. Thomson Paris, DOE/USDA, ORISE participant, sample collection.

This research was supported in part by an appointment to the Agricultural Research Service (ARS) Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA). ORISE is managed by ORAU under DOE contract number DE-SC0014664. All opinions expressed in this paper are the author's and do not necessarily reflect the policies and views of USDA, ARS, DOE, or ORAU/ORISE.

Funding in part: USDA, National Institutes of Food and Agriculture

USDA, NIFA, National Institute of Food and Agriculture, Citrus Greening award #2015-70016-23028, "*Developing an Infrastructure and Product Test Pipeline to Deliver Novel Therapies for Citrus Greening Disease*", Lead PI: Dr. S. Brown, Kansas State University, KS, USA.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Chapman, A.D. *Numbers of Living Species in Australia and the World*, 2nd edition. Australian Biodiversity Information Services **2009**, pg. 5-24, ISBN 978 0 642 56861 8.
2. Asgari, S. Johnson, K.N.J. *Insect Virology*. Caister Academic Press **2010**, pg. 453, ISBN 1904455719.
3. Wu, Q.; Luo, Y.; Lu, R.; Lau, N.; Lai, E.C.; et al. Virus discovery by deep sequencing and assembly of virus-derived small silencing RNAs. *Proceedings of the. National Academy of Science, USA;* **2010**; 107: 1606–1611. doi:10.1073/pnas.0911353107.
4. Russell, D.N; Qureshi, J.A; Halbert, S.E; Stansly, P.A. Host suitability of citrus and *Zanthoxylum* spp. For *Leuronota fagarae* Burckhardt and *Diaphorina citri* Kuwayama (Hemiptera: Psylloidea), *Florida Entomologist* **2014**, 97(4):1481-1492, doi:10.1653/024.097.0424.
5. King, A.M.Q.; Adams, M.J.; Carstens, E.B.; Lefkowitz, E.J; *Virus Taxonomy* 1st Edition, Elsevier **2012**, pg. 855-880, ISBN: 9780123846853.
6. MacLachlan, J.N.; Dubovi, E.J. *Fenner's Veterinary Virology* (Fifth Edition), Elsevier **2017**, pg. 477-495, ISBN: 9780128011706.
7. Ryabov, E.V. Invertebrate RNA virus diversity from a taxonomic point of view, *Journal of Invertebrate Pathology* **2017**, 147: 37-50. doi:10.1016/j.jip.2016.10.002.
8. Cicero, J.M.; Brown, J.K. A stationary tweezer platform for high throughput dissections of minute arthropods and extirpation of their minute organs. *MethodsX* **2021**; 8: 1-9. doi:10.1016/j.mex.2021.101317.
9. Okonechnikov, K.; Golosova, O.; Fursov, M.; UGENE team. Unipro UGENE: a unified bioinformatics toolkit. *Bioinformatics* **2012**; 28:1166-1167. doi:10.1093/bioinformatics/bts091.
10. Gasteiger, E.; Gattiker, A.; Hoogland, C.; Ivanyi, I.; Appel, R.D.; Bairoch, A. ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res.* **2003**, 32: 3784-3788. doi: 10.1093/nar/gkg563.
11. National Center for Biotechnology Information (NCBI) [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [2021 Jun 04].

12. Nouri, S.; Salem, N.; Nigg, J.C.; Falk, B.W. Diverse array of new viral sequences identified in worldwide populations of the Asian citrus psyllid (*Diaphorina citri*) using viral metagenomics. *Journal of virology* **2015**, 90(5):2434–2445, doi:10.1128/JVI.02793-15.
13. Upadhyay, S.K.; Sharma, S.; Singh, H.; Dixit, S.; Kumar, J.; et al. Whitefly genome expression reveals host-symbiont interaction in amino acid biosynthesis. *PLoS ONE* **2015**, 10(5):e0126751. https://doi.org/10.1371/journal.pone.0126751
14. Kumar, S.H.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **2018**; 35: 1547-1549. doi:10.1093/molbev/msy096.
15 Berman, J.J. *Taxonomic Guide to Infectious Diseases*, Academic Press **2012**, pg. 237-246, ISBN: 9780124159136.
16. Johansson, H.; Dhaygude, K.; Lindström, S.; Helanterä H.; Sundström, L. et al. A metatranscriptomic approach to the identification of microbiota associated with the ant *Formica exsecta*. *PLoS ONE* **2013**, 8(11): e79777. https://doi.org/10.1371/journal.pone.0079777.
17. Wang, J.; Roose, M.; Ramadugu, C.; Lee, R.; Manjunath, K.; Lin, H. et al. Preliminary evaluation of the single-tree, huanglongbing find in California. *Journal of Citrus Pathology* **2014**, 1(1): 2313-5131.
18. Dala-Paula, B.M.B.; Plotto, A.; Bai, J.; Manthey, J.A.; Baldwin, E.A.; Ferrarezi, R.S.; Gloria, M.B.A. Effect of huanglongbing or greening disease on orangejJuice quality, a review. *Frontiers in Plant Science* **2019**, 9:1976. doi:10.3389/fpls.2018.01976.
19. Lacey, L.A.; Grxywacz, D.; Shapiro-Ilan, D.I.; Frutos, R.; Brownbridge, M.; Goettel, M.S. **2015**. Insect pathogens as biological control agents: Back to the future. *Journal of Invertebrate Pathology* 132: 1–41. doi.org/10.1016/j.jip.2015.07.009.
20. Kolliopoulou, A.; Taning, C.N.T.; Smagghe, G.; Swevers, L. **2017**. Viral delivery of dsRNA for control of insect agricultural pests and vectors of human disease: Prospects and challenges. *Frontiers in Physiology*. 8:399. doi:10.3389/fphys.2017.00399.
21. Saha, S.; Hunter, W.; Mueller, L. **2019**. AgriVectors: a systems biology portal for plant pathosystems and arthropod vectors of plant diseases. *Proceedings of the APS Annual Conference*, August 3-7, Clevland, OH, U.S.A. https://www.apsnet.org/meetings/annual/meetingarchives/planthealth2019/Documents/Abstracts/aps2019ab171.htm

Supplementary Material

Table S1. Accession numbers for phylogeny tree comparing LfPLV RdRp protein sequence to other arthropods.

Name	Abbreviation	Accession Number
Diaphorina citri picorna-like virus	DcPLV	ALJ52073.1
Riptortus pedestris virus-2	RpV-2	QDE12516.1
Bemisia tabaci	Bt	AKC57283.1
Vespa velutina Moku virus	VvMV	ATY36108.1
Darwin bee virus 3	DBV	AWK77848.1
Helicoverpa armigera iflavirus	HaIFV	YP_009344960.1
Antheraea pernyi iflavirus	ApIFV	YP_009002581.1
Psammotettix alienus iflavirus 1	PaIFV-1	YP_009553259.1
Lymantria dispar iflavirus 1	LdIFV-1	YP_009047245.1
Slow bee paralysis virus	SBPV	ADI46683.1
Bombyx mori iflavirus	BmIFV	YP_009162630.1
Moran virus	Mv	QED21536.1
Nilaparvata lugens honeydew virus 1	NlHV-1	YP_009505599.1
Thaumetopoea pityocampa iflavirus 1	TpIFV	YP_009116875.1
Tribolium castaneum iflavirus	TcIFV	AUE23905.1
Scaphoideus titanus iflavirus 1	StIFV	QIJ56901.1
Varroa destructor virus 1	VdV-1	AGO86045.1
Deformed Wing Virus	DWV	AGA20423.1
Acute Bee Paralysis Virus	ABPV	NP_066241.1
Cricket Paralysis Virus	CPV	NP_647481.1
Leuronota fagarae picorna-like virus	LfPLV	

Figure S1. Predicted protein translation of LfPLV RdRp coding region, https://web.expasy.org/translate/

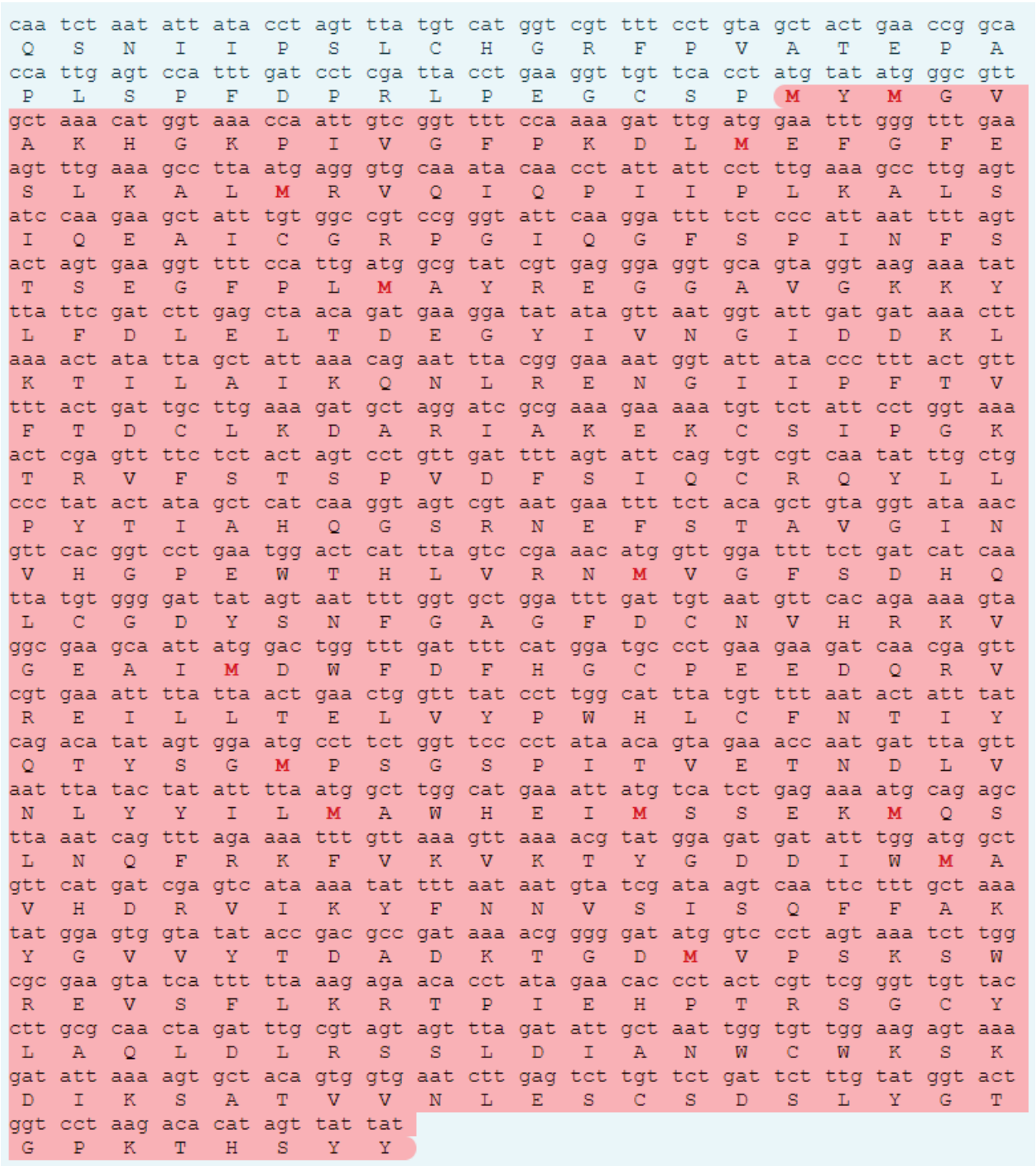

Figure S2. Predicted RdRp protein sequence alignment between LfPLV and DcPLV (NCBI, BLASTp).

Score: 634 bits (1634) Expect: 0.0 Method: Compositional matrix adjust.

Identities: 301/466(65%) Positives: 369/466(79%) Gaps:3/466(0%)

```
LfPLV  1     QSNIIPSLCHGRFPVATEPAPLSPFDPRLPEGCSPMYMGVAKHGKPIVGFPKDLMEFGFE  60
            +S+I+PSLCHG F V TEPAPLS  DPRLP G  PM +GV KHGKPI GFP DL++FGFE
DcPLV  1414  KSSIVPSLCHGIFEVMTEPAPLSRSDPRLPPGTDPMILGVNKHGKPIRGFPSDLIKFGFE  1473

LfPLV  61    SLKALMRVQIQPIIPLKALSIQEAICGRPGIQGFSPINFSTSEGFPLMAYREGGAVGKKY  120
            SL++++RV+++P+I +   S++EAI GR GI GF+ IN  +SEGFPL A +  G  GKKY
DcPLV  1474  SLRSIVRVRVKPLIKVTPTSLEEAILGRAGIGGFASINMHSSEGFPLSALKPPGVTGKKY  1533

LfPLV  121   LFDLELTDEGYIVNGIDDKLKTILAIKQNLRENGIIPFTVFTDCLKDARIAKEKCSIPGK  180
            LFD +L  +   + GID+ LKTI++IK  LR+ G +PFTVFTDCLKDARIAKEKC IPGK
DcPLV  1534  LFDCDLDKKE--LYGIDENLKTIMSIKDGLRKKGKVPFTVFTDCLKDARIAKEKCRIPGK  1591

LfPLV  181   TRVFSTSPVDFSIQCRQYLLPYTIAHQGSRNEFSTAVGINVHGPEWTHLVRNMVGFSDHQ  240
            TR+FS SPVDFSIQ RQY LPYT+AHQ SR +FS+AVGINV+G EW+ LV  M+ FS +Q
DcPLV  1592  TRIFSVSPVDFSIQFRQYFLPYTVAHQNSRWDFSSAVGINVNGVEWSVLVGKMIRFSPYQ  1651

LfPLV  241   LCGDYSNFGAGFDCNVHRKVGEAIMDWFDFHGC-PEEDQRVREILLTELVYPWHLCFNTI  299
            LCGDYSNFGAGFD  VHR VGE ++DWF F+G   EE++ +R ++L ELVYPWHLC + +
DcPLV  1652  LCGDYSNFGAGFDEEVHRMVGEILIDWFKFNGDDSEENETIRRVMLHELVYPWHLCKDIL  1711

LfPLV  300   YQTYSGMPSGSPITVETNDLVNLYYILMAWHEIMSSEKMQSLNQFRKFVKVKTYGDDIWM  359
            YQT SGMPSGSPITVETNDLVNLYYILM W +IM   K+ +L +F K+V+VKTYGDDIWM
DcPLV  1712  YQTVSGMPSGSPITVETNDLVNLYYILMMWFDIMRPLKLHTLKKFEKYVRVKTYGDDIWM  1771

LfPLV  360   AVHDRVIKYFNNVSISQFFAKYGVVYTDADKTGDMVPSKSWREVSFLKRTPIEHPTRSGC  419
            AVH  VI+YFNN++IS+ FA+YGV YTDADK G   P +SW EVSFLKRTP  HPTR
DcPLV  1772  AVHPDVIEYFNNMTISKAFAQYGVEYTDADKKGMDKPYRSWEEVSFLKRTPKVHPTRLNH  1831

LfPLV  420   YLAQLDLRSSLDIANWCWKSKDIKSATVVNLESCSDSLYGTGPKTH  465
            +LA LDL S+LDIANWC++S D+  +T+VNLE+CSD +YG GP+ H
DcPLV  1832  FLAALDLNSTLDIANWCYESNDMAVSTLVNLEACSDMMYGHGPEKH  1877
```

Figure S3. Phylogenetic comparison between LfPLV RdRp nucleotide sequence and four of the closest related sequences, Neighbor Joining, NJ.

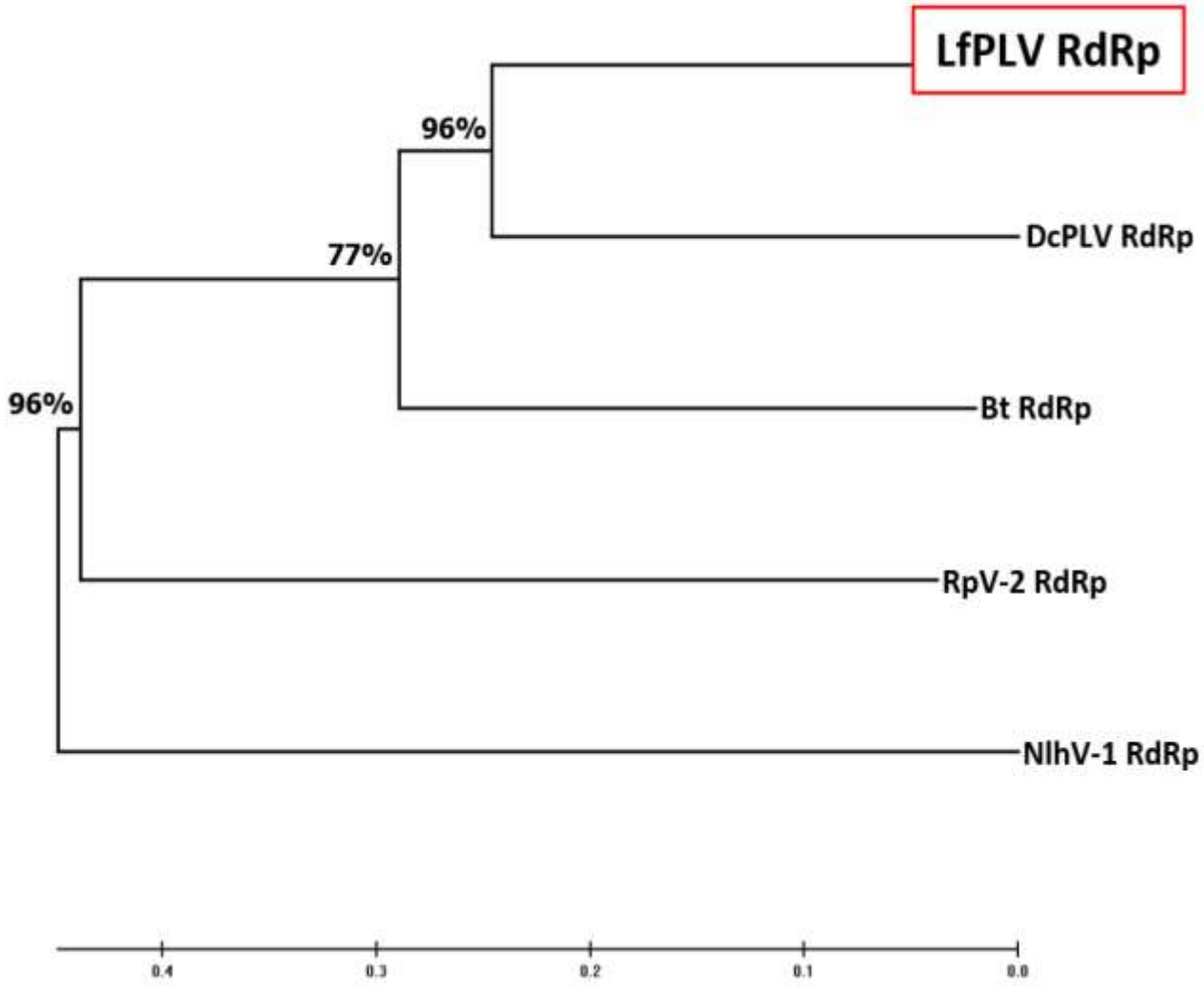

Table S2. Accession numbers for phylogeny tree comparing LfPLV RdRp nucleotide sequence to the TOP four closest related viral sequences.

Name	Abbreviation	Accession Number
Diaphorina citri picorna-like virus	DcPLV	KT698837.1
Riptortus pedestris virus-2	RpV-2	MN078225.1
Bemisia tabaci	Bt	KJ994272.1
Nilaparvata lugens honeydew virus 1	NlhV-1	NC_038302.1
Leuronota fagarae picorna-like virus	LfPLV	Agrivectors 2021_LfPLV-FL