

Picornavirus-like Virus Discovered in Wild Lime Psyllid *Leuonota fagarae* Burckhardt (Hemiptera: Psylloidea)

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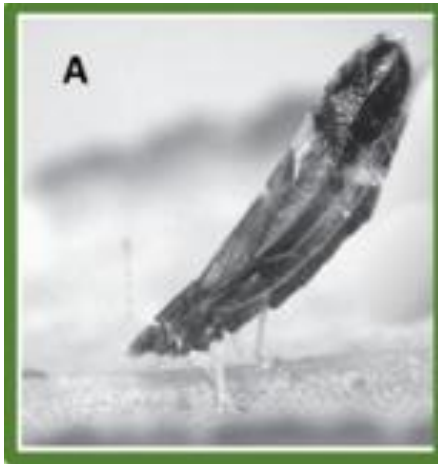
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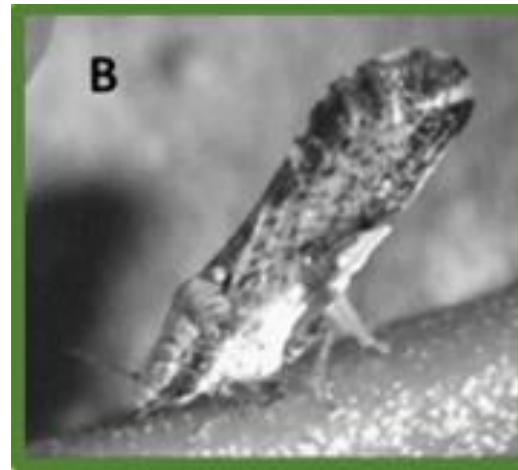
Douglas S. Stuehler 1,2*

Background

- ▶ ***Diaphorina citri* (Asian citrus psyllid)**
 - ▶ Is a vector of the bacterium, *Candidatus Liberibacter asiaticus*, agent of Huanglongbing (HLB).
 - ▶ HLB is the single most devastating disease of citrus trees, existing as a threat to world citrus production.
- ▶ ***Leuronota fagarae* (Wild lime psyllid)**
 - ▶ Invasive species in the state of Florida, U.S.A., with South American origin.
 - ▶ Currently uncharacterized vector capabilities, is not a pest, or known vector of pathogens.



Leuronota fagarae



Diaphorina citri

Russell, Qureshi, Halbert, Stansly, 2014.
Florida Entomol. 97 (4): 1481-1492.

Research Goal

- Produce genome & transcriptome for bioinformatic analyses for comparative characterization of *Leuronota fagarae* with *Diaphorina citri*, in Florida, USA.
 - Further insight into the diverse vector capabilities of *Leuronota fagarae*.
 - Identification of psyllid pathogens, and the microbiome.
 - Accelerate pathogen discovery, for use in the management of psyllid vectors.



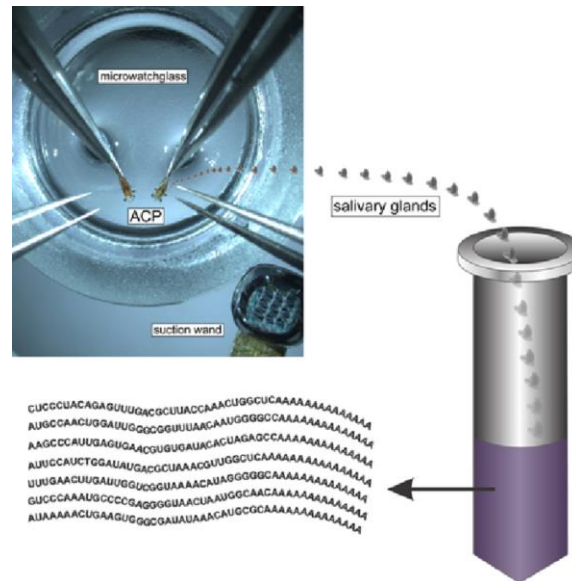
Mating *Leuronota fagarae*

Russell, Qureshi, Halbert, Stansly, 2014.
Florida Entomol. 97 (4): 1481-1492.



Data Collection

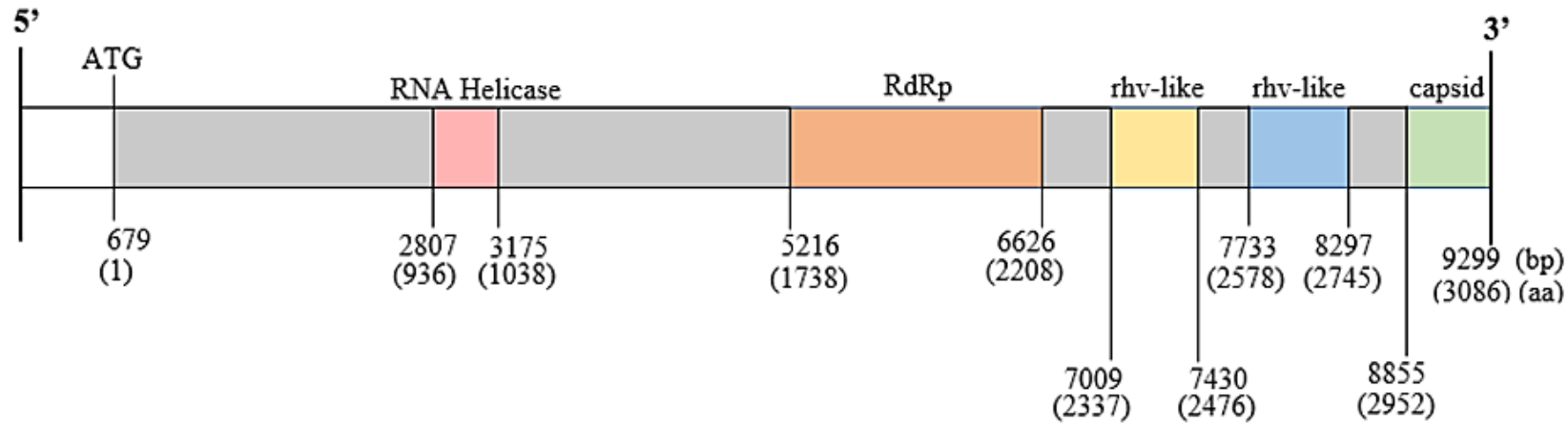
- ▶ *Leuronota fagariae* colonies were maintained at Department of Plant Industry, DPI, Gainesville, Florida, and at the University of Florida, Indian River Research and Education Center in glass houses maintained at 27 °C and 60% RH.
- ▶ Salivary glands were dissected from 900 live adult psyllids, immediately placed into TRIzol™-LS Reagent.
- ▶ The cDNA library was constructed from the salivary gland extractions and sequenced at the University of Florida, Core Genomics Facility, Gainesville, FL, USA.



Bioinformatic Analysis

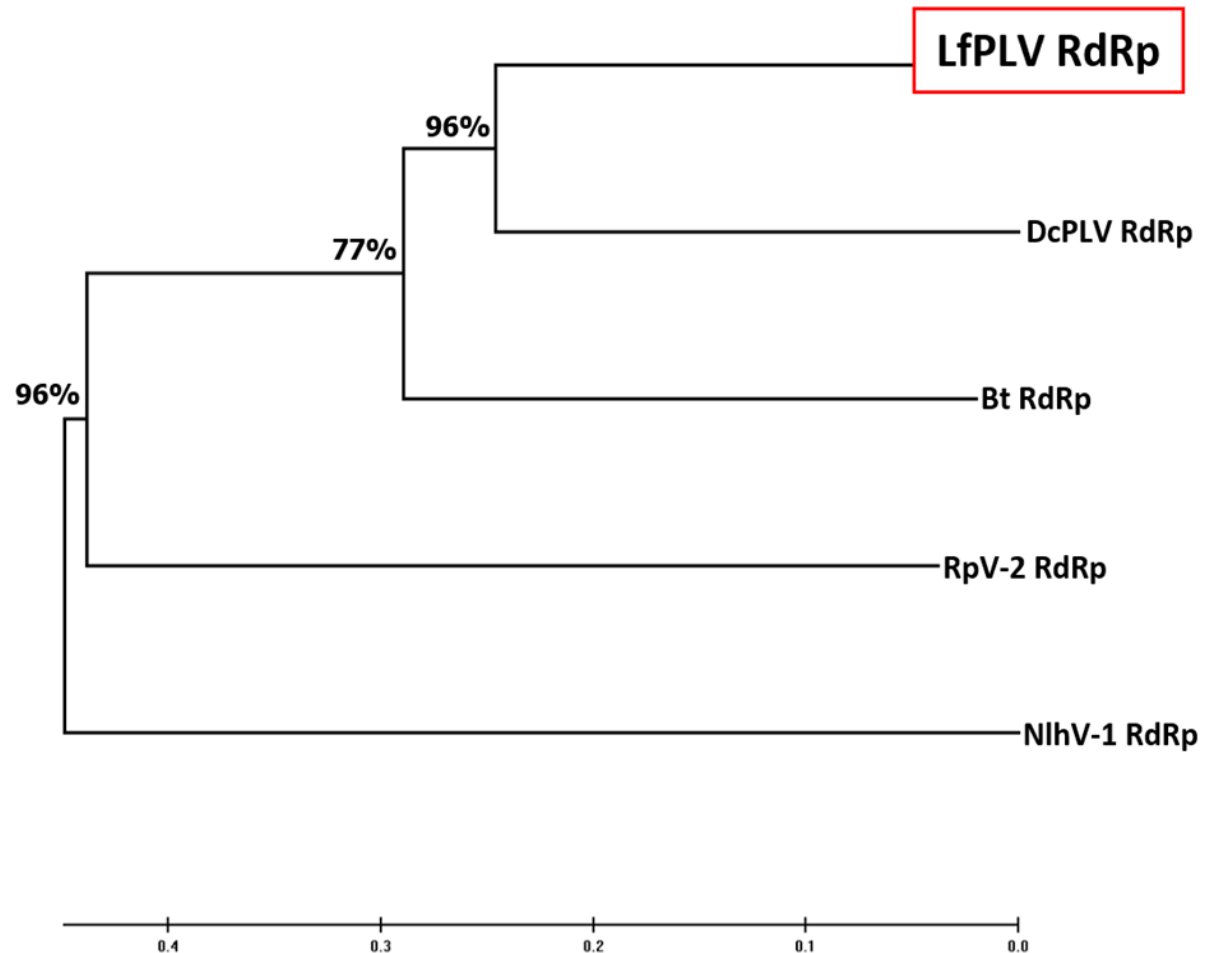
- ▶ Reads were assembled with the TRINITY assembly program.
- ▶ Assembled orthologs were translated and subject to BLASTp analysis.
 - ▶ Top alignments identified significant E-values to invertebrate picorna-like viruses.
 - ▶ Top alignment was to *Diaphorina Citri* picorna-like virus.
- ▶ Further analysis revealed the near-complete CAP3 genome assembly of a newly identified viral species, existing in *Leuronota fagarae*, Florida-isolate.
 - ▶ RdRp sequences were identified from 20 other closely related insect infecting picornaviruses and aligned with ClustalW.
 - ▶ The resulting alignment was used to build a Neighbor-Joining phylogenetic tree.

Gene Map



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

Phylogenetic Analysis



- Phylogenetic tree RdRp amino acid sequence alignments, Top 4, depicting evolutionary relations of five RdRp nucleotide sequence identified through multiple alignments using ClustalW and translation on the ExPASy server. LfPLV was most closely related to *Diaphorina citri-Picornavirus-like Virus*.

Alignments

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 | KSSIVPSLCHGIFEVMTTEPAPLSRSDPRLPPGTDPMILGVNKHGKPIRGFPSDLIKFGFE | 1473 |
| Query | 61 | SLKALMRVQIQPIIPLKALSIQEAIICGRPGIQGFSPINFSTSEGFPLMAYREGGAVGKKY | 120 |
| | | SL++++RV+++P+I + S++EAI GR GI GF+ IN +SEGFPL A + G GKKY | |
| Sbjct | 1474 | SLRSIVRVRVKPLIKVTPPTSLEEAILGRAGIGGFASINMHSSEGFPLSALKPPGVTGKKY | 1533 |
| Query | 121 | LFDLELTDEGYIVNGIDDKLKTILAIKQNLRENGIIPFTVFTDCLKDARIAKEKCSIPGK | 180 |
| | | LFD +L + + GID+ LKTI++IK LR+ G +PFTVFTDCLKDARIAKEK IPGK | |
| Sbjct | 1534 | LFDCDLDKKE--LYGIDENLKTIMSIKDGLRKKGKVPFTVFTDCLKDARIAKEKCRIPGK | 1591 |
| Query | 181 | TRVFSTSPVDFSIQCRQYLLPYTIAHQGSRNEFSTAVGINVHGPEWTHLVRNMVGFSDHQ | 240 |
| | | TR+FS SPVDFSIQ RQY LPYT+AHQ SR +FS+AVGINV+G EW+ LV M+ FS +Q | |
| Sbjct | 1592 | TRIFSVSPVDFSIQFRQYFLPYTVVAHQNSRWDFSSAVGINVNGVEWSVLVGKMIRFSPYQ | 1651 |
| Query | 241 | LCGDYSNFGAGFDCNVHRKVGEAIMDWFDFHGC-PEEDQRVREILLTELVPWHLCFNFI | 299 |
| | | LCGDYSNFGAGFD VHR VGE ++DWF F+G EE++ +R ++L ELVYPWHLC + + | |
| Sbjct | 1652 | LCGDYSNFGAGFDEEVHRMVGEILIDWFKFNGDDSEENETIRRVMHELVPWHLCKDIL | 1711 |
| Query | 300 | YQTYSGMPSPITVETNDLVNLYYILMAWHEIMSSEKMQSLNQFRKFVKVKTYYGDDIWM | 359 |
| | | YQT SGMPSPITVETNDLVNLYYILM W +IM K+ +L +F K+V+VKTYGDDIWM | |
| Sbjct | 1712 | YQTVSGMPSPITVETNDLVNLYYILMMWFDIMRPLKLHTLKKFEKYVRVKTYGDDIWM | 1771 |
| Query | 360 | AVHDRVIKVFNNVSIQFFAKYGVVYTDADKTGDMVPSKSWREVSFLKRTPIEHPTRSGC | 419 |
| | | AVH VI+YFNN++IS+ FA+YGV YTDADK G P +SW EVSFLKRTP HPTR | |
| Sbjct | 1772 | AVHPDVIEYFNNMTISKAFAYGVEYTDADKKGMKPYRSWEEVSFLKRTPKVHPTRLNH | 1831 |
| Query | 420 | YLAQLDLRSSLDIANWCWKSVDIKSATVVNLESCSDSLYGTGPKTH 465 | |
| | | +LA LDL S+LDIANWC++S D+ +T+VNLE+CSD +YG GP+ H | |
| Sbjct | 1832 | FLAALDLNSTLDIANWCYESNDMAVSTLVNLEACSDMMYGHGPEKH 1877 | |

► Alignment of LfPLV and DcPLV RdRp amino acid sequences in NCBI BLASTp.

► Top row of each line is the LfPLV RdRp amino acid sequence.

► Bottom rows are DcPLV RdRp.

Conclusion

- ▶ We report on the discovery of a new picorna-like virus infecting *Leuronota fagarae* (Hemiptera: Psylloidea), with the proposed name: “*Leuronota fagarae* picorna-like virus, LfPLV-FL, Florida isolate, 2021.”
- ▶ The research focus is to identify viruses that infect psyllids, which may have use as expression vectors for RNAi biopesticides to control psyllid vectors.
- ▶ Further bioinformatic analyses and *in vivo* studies will provide information for the final classification of LfPLV taxonomy.
- ▶ The discovery of viruses that infect psyllids provides a valuable resource that can be used for psyllid pest management.
- ▶ Ultimately development of biopesticides for controlling the Asian citrus psyllid, *D. Citri*, and other psyllid vectors of economically important pathogens is our goal.

Organizations and Acknowledgements

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