

**Comparative genome characteristics  
Cotton leaf roll dwarf-like  
poleroviruses**

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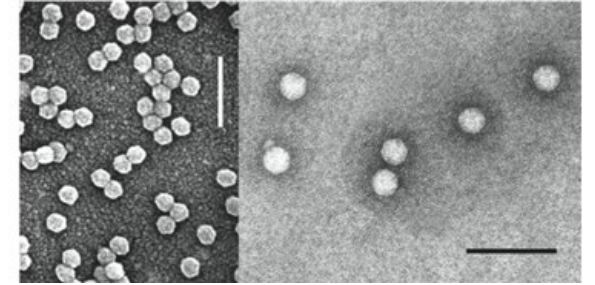
# September 25, 2017 symptoms courtesy: Dr. Kathy Lawrence



# Luteoviridae; genus, *Polerovirus*

**Particles** icosahedral ~23 nm diam; T=3 capsid structure

**Genome:** Linear positive sense RNA of 5.6-6.0 kb



## **Aphid transmission:**

-Major coat protein 21–23 kDa encoded by ORF3; Minor CP: “readthrough” = C-term fusion of ORF3 and contiguous ORF5; Readthrough protein associated with aphid transmission

-Persistent, circulative; Vector specificity often strict

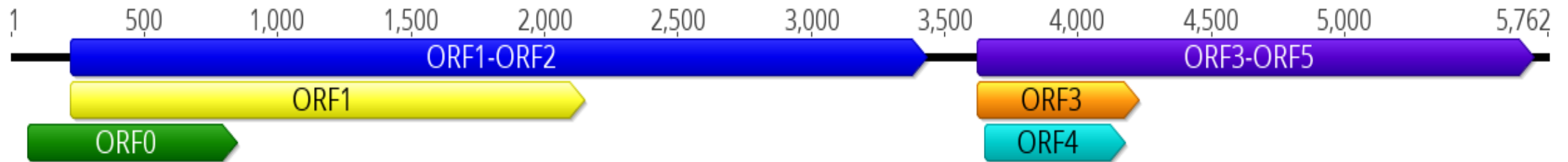
-Cotton aphid holocyclic in US –primary hosts: *Catalpa*, *Rhamnus*, and *Hibiscus*

-Phloem-limited



**Symptoms:** Phloem necrosis spreading from inoculated sieve elements; causes symptoms by inhibiting translocation (reddening due to P/ other nutrient deficiencies), slowed plant growth, and loss of chlorophyll, yellowing and dwarfing of infected leaves /whole plant.

# Poliovirus genome organization and gene functions



- ORF0 (P0): silencing suppressor
- ORF1 (P1): VPg precursor (?)
- ORF1-ORF2 (P2): RNA dependent RNA polymerase
- ORF3 (P3): coat protein
- ORF3a (P3a) long distance movement
- ORF4 (P4): movement protein
- ORF3-ORF5 (P3-P5): aphid transmission protein

## Characteristic ‘polerovirus’ symptoms:

Phloem necrosis spreading from inoculated sieve elements; causes symptoms by inhibiting translocation (reddening due to P/ other nutrient deficiencies), slowed plant growth, and loss of chlorophyll, resulting in yellowing and dwarfing of infected leaves and/or whole plant.

Alabama-Aug 2017



Alabama Aug 2018  
cotton



CLRDV in cotton in Brazil



CLRDV in cotton in India



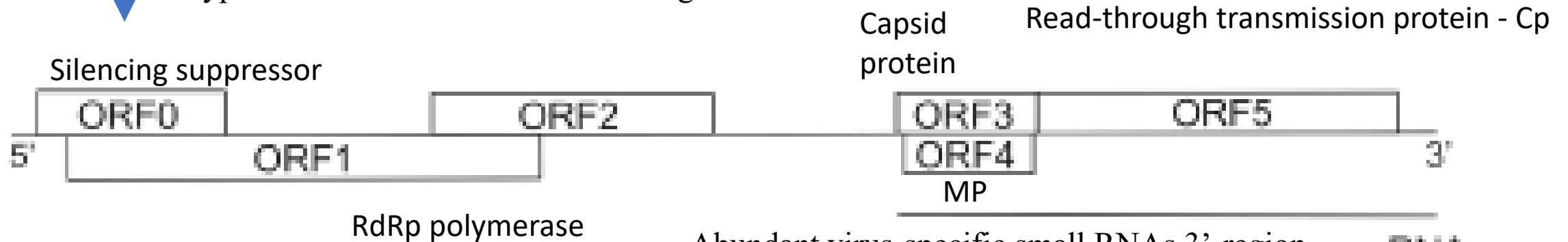
**Taxonomy: Species demarcation**  
10% divergence at AA level for any gene

**Species demarcation criteria for the Luteoviridae / genus, *Polerovirus***

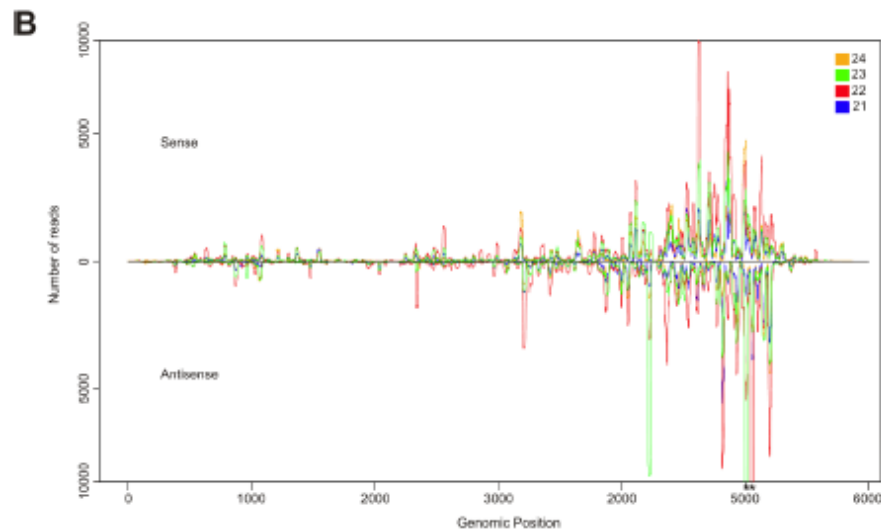
- Criteria used to demarcate species of the genus include:
- Differences in breadth and specificity of host range
- Failure of cross-protection in either one-way or two-way relationships
- Differences in serological specificity with discriminatory polyclonal or monoclonal antibodies
- Differences in amino acid sequence identity of any gene product of greater than 10%.

# Host gene silencing: Small RNAs target viral dsRNA(replicative form)

↓ Viral suppressor of host gene silencing:  
'Atypical'-variant overcame resistance gene



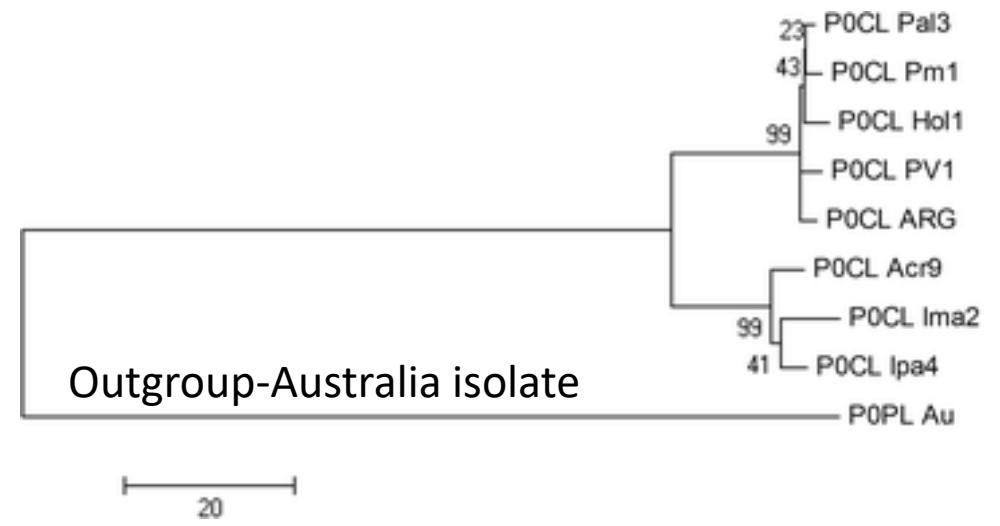
Abundant virus-specific small RNAs 3'-region  
Especially ORF-5 ~4300-5300 **sgRNA**



- Virus-derived RNAs 21-24-nt , entire genome sense and anti-sense strands
- Atypical symptoms: highly variable; multiple virus variants in BZ
- P0 protein mediates the decay of ARGONAUTE proteins, a key component of the RNA silencing machinery (Cascardo et al. Virology Journal (2015) 12:123).

# Range of AA% divergence P0 suppressor 10% any gene = distinct spp

Cascardo et al. Virology Journal (2015) 12:123



**Table 2** Percentage of amino acid identity among the viral isolates used in the study

P0 <sup>CL</sup> -Pal3	X								
P0 <sup>CL</sup> -Pm1	98.88	X							
P0 <sup>CL</sup> -PV1	98.51	98.14	X						
P0 <sup>CL</sup> -Hol1	98.51	98.14	97.76	X					
P0 <sup>CL</sup> -ARG	98.88	98.51	98.14	98.14	X				
P0 <sup>CL</sup> -Ima2	86.24	85.87	86.61	85.5	86.24	X			
P0 <sup>CL</sup> -Ipa4	88.1	87.73	87.73	87.36	87.36	96.28	X		
P0 <sup>CL</sup> -Acr9	88.1	87.73	87.73	87.36	88.1	95.53	97.02	X	
P0 <sup>PL</sup> -AU	18.21	18.21	17.84	18.21	18.58	17.84	18.58	18.58	X
	P0 <sup>CL</sup> -Pal3	P0 <sup>CL</sup> -Pm1	P0 <sup>CL</sup> -PV1	P0 <sup>CL</sup> -Hol1	P0 <sup>CL</sup> -ARG	P0 <sup>CL</sup> -Ima2	P0 <sup>CL</sup> -Ipa4	P0 <sup>CL</sup> -Acr9	P0 <sup>PL</sup> -AU





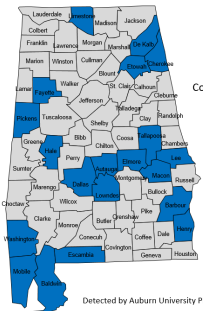
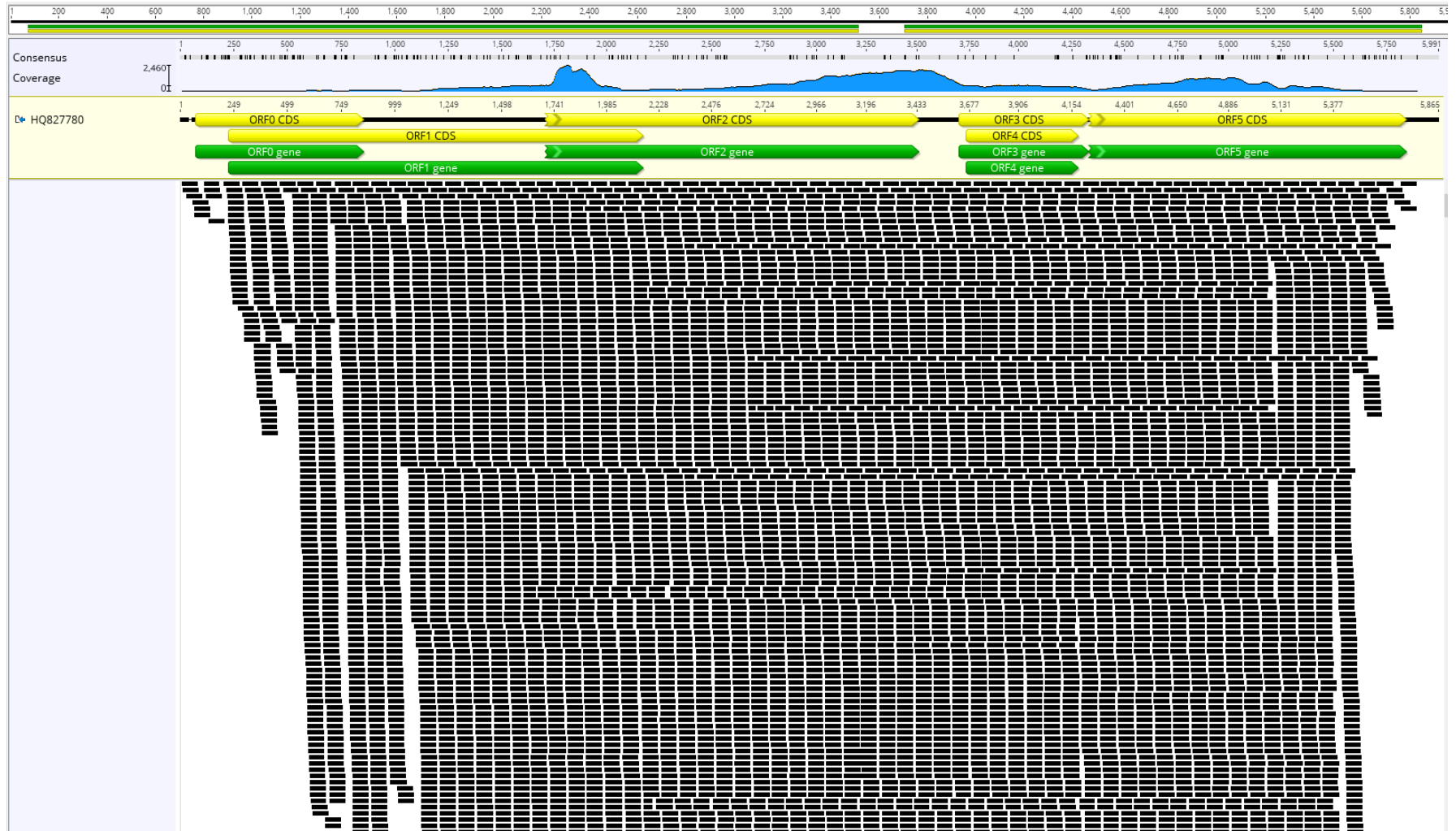
# How do we know what virus it is?

## Cotton samples from Alabama submitted for Illumina RNA sequencing 2017 and 2018

Isolate name	Sample name	County	Collection date
F1	F1	Barbour	September 2017
C1*	EVS 9/19	Macon	September 2018
C3	Back field DP 1646 top 1-6	Barbour	September 2018

\* Best reads coverage

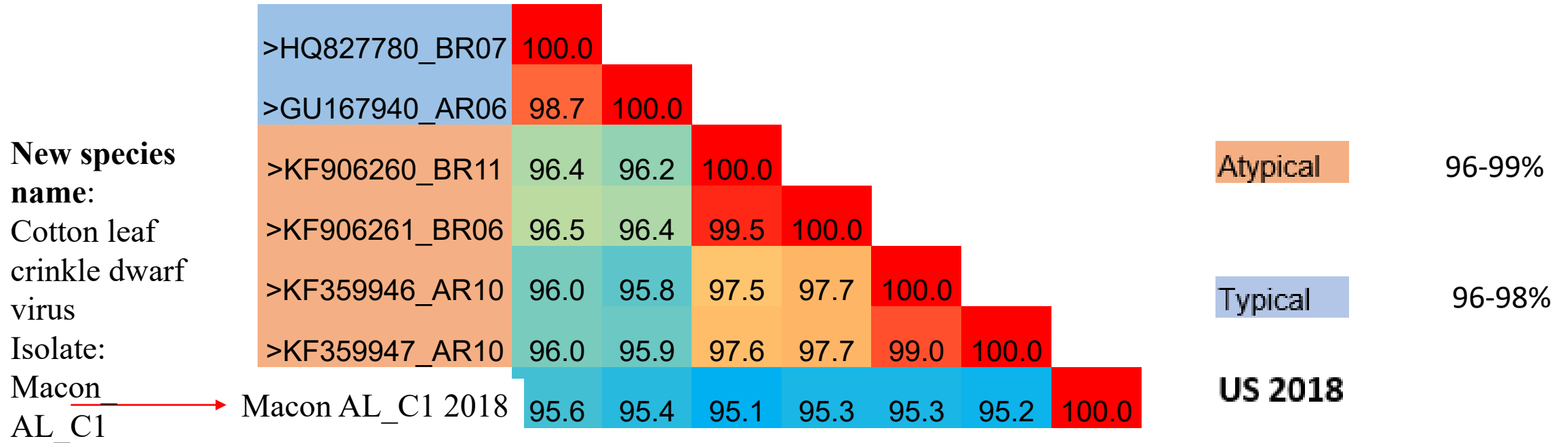
# CLRDV-US18 reference-guided assembly using Illumina reads from 2018



Cotton Blue Disease  
Cotton leafroll dwarf virus (CLRDV)  
December 6, 2018

Detected by Auburn University Plant Diagnostic Lab

# Complete genome comparison: nucleotide sequence identity (%) ~ same divergence between all three groups

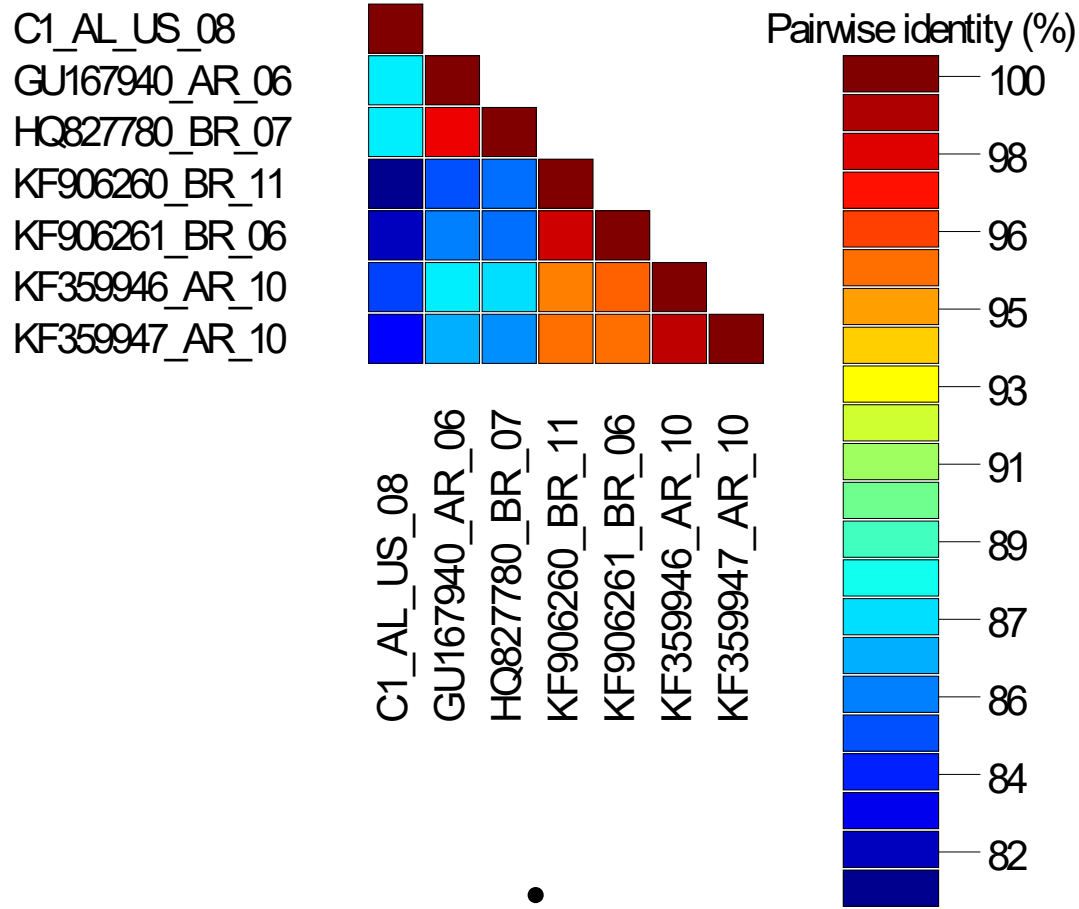


Sequence Demarcation Tool (SDT) v. 1.2



# ORF 0 amino acid pairwise identity

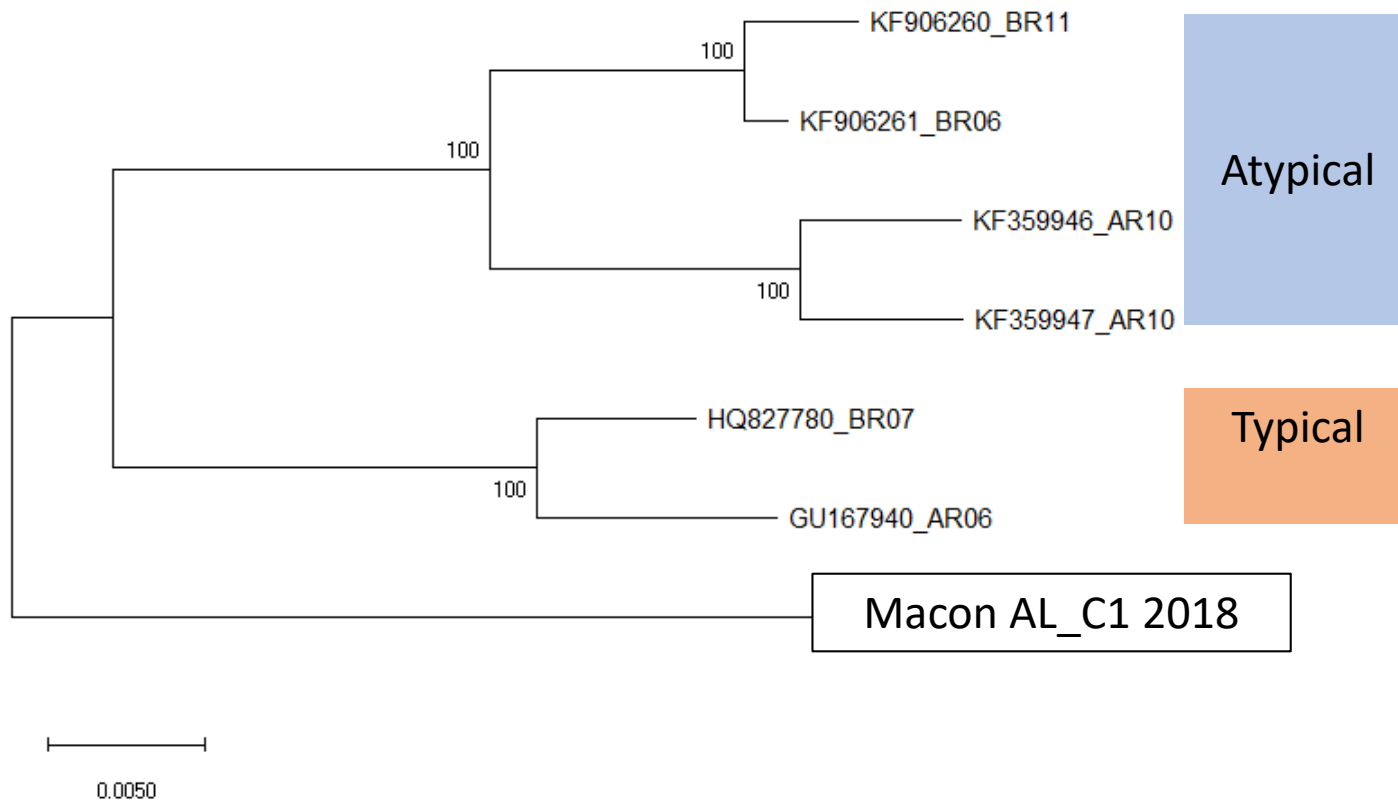
- 82.4-88.5% amino acid sequence pairwise identity
- Other ORFs are ~90-100 %



By taxonomic criteria – **new species**

# CLRDV & ACLRDV, Macon C\_1 isolate

## Phylogenetic analysis confirms 3 separate species



- MEGAX software
- Maximum Likelihood (ML)
- 1000 bootstrap iterations

### Three clades

- Atypical
- Typical
- USA Macon AL\_C1 2018

# Pairs of RT-PCR primers recommended for virus detection in 2018 cotton season

Primers	Primer sequence	Target	Annealing temperature	Expected size	Reference
CLR DV3675F	CCACGTAGRCGCAACAGGCGT	CLR DV specific	56 for 10 s and 62 fo 20	310	Sharman et al. 2015
Pol3982R	CGAGGCCTCGGAGATGAACT	CLR DV, CBT V, CABYV, CpCSV, SbDV			
CLR DV_674F	CCGTAGCGGTCATCGTCTTT	CLR DV specific	55	733	Avelar et al. 2018
CLR DV_1407R	TAACCCTGACACAGTGGGGA	CLR DV specific			

– 2019 (*Plant Dis. In press*)

## **First report of Cotton blue disease in the United States**

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