

Comparative genome characteristics Cotton leaf roll dwarf-like poleroviruses

Judith K. Brown
Plant Sciences
The University of Arizona
Tucson, AZ 85721 USA
jbrown@ag.arizona.edu

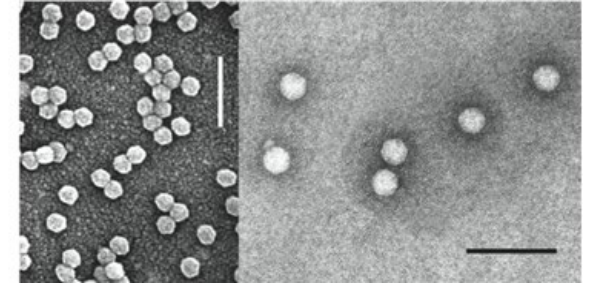
September 25, 2017 symptoms courtesy: Dr. Kathy Lawrence



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

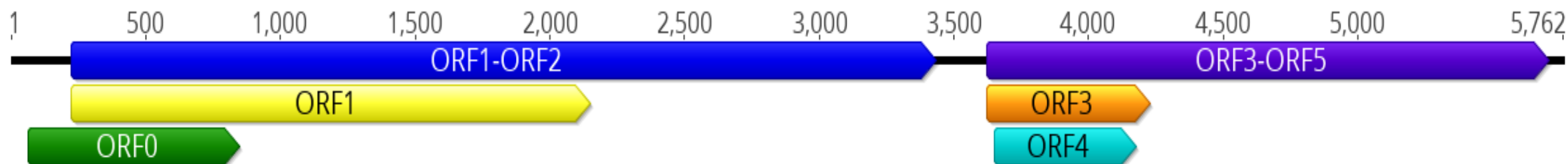
-Cotton aphid holocyclic in US –primary hosts: *Catalpa*, *Rhamnus*, and *Hibiscus*

-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.

Polerovirus 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



CLRDTV in cotton in Brazil



CLRDTV in cotton in India



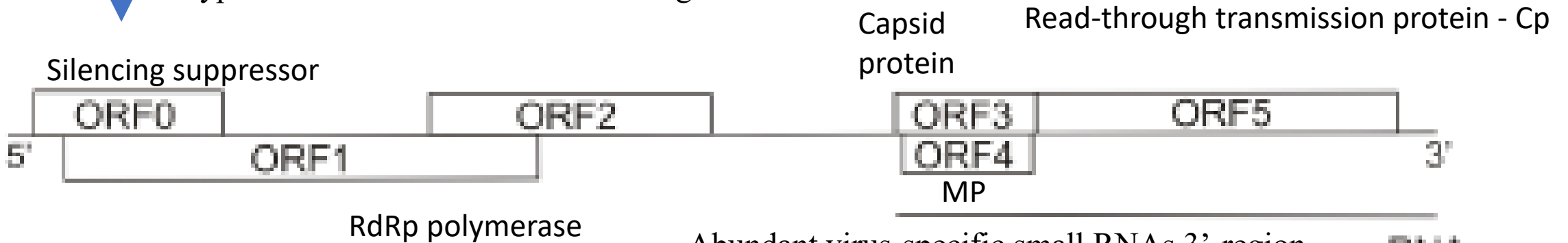
Taxonomy: Species demarcation
10% divergence at AA level for any gene

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

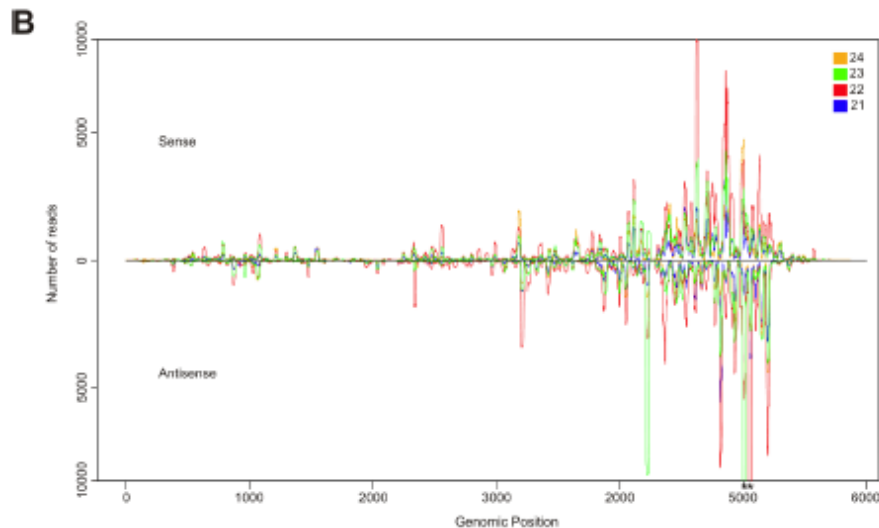
- Criteria used to demarcate species of the genus include:
- Differences in breadth and specificity of host range
- Failure of cross-protection in either one-way or two-way relationships
- Differences in serological specificity with discriminatory polyclonal or monoclonal antibodies
- Differences in 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

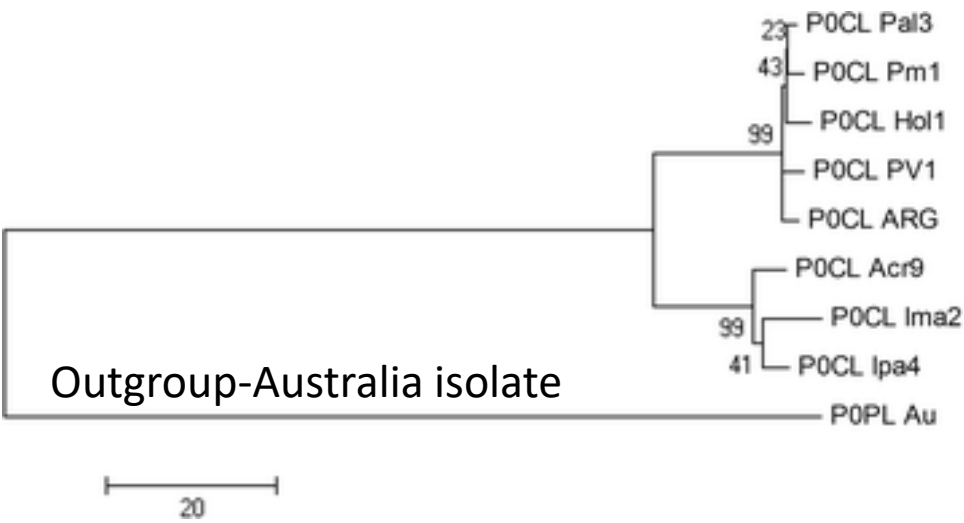


Table 2 Percentage of amino acid identity among the viral isolates used in the study

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



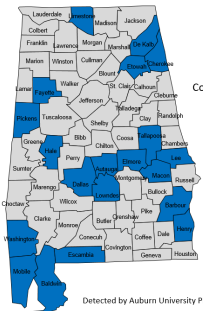
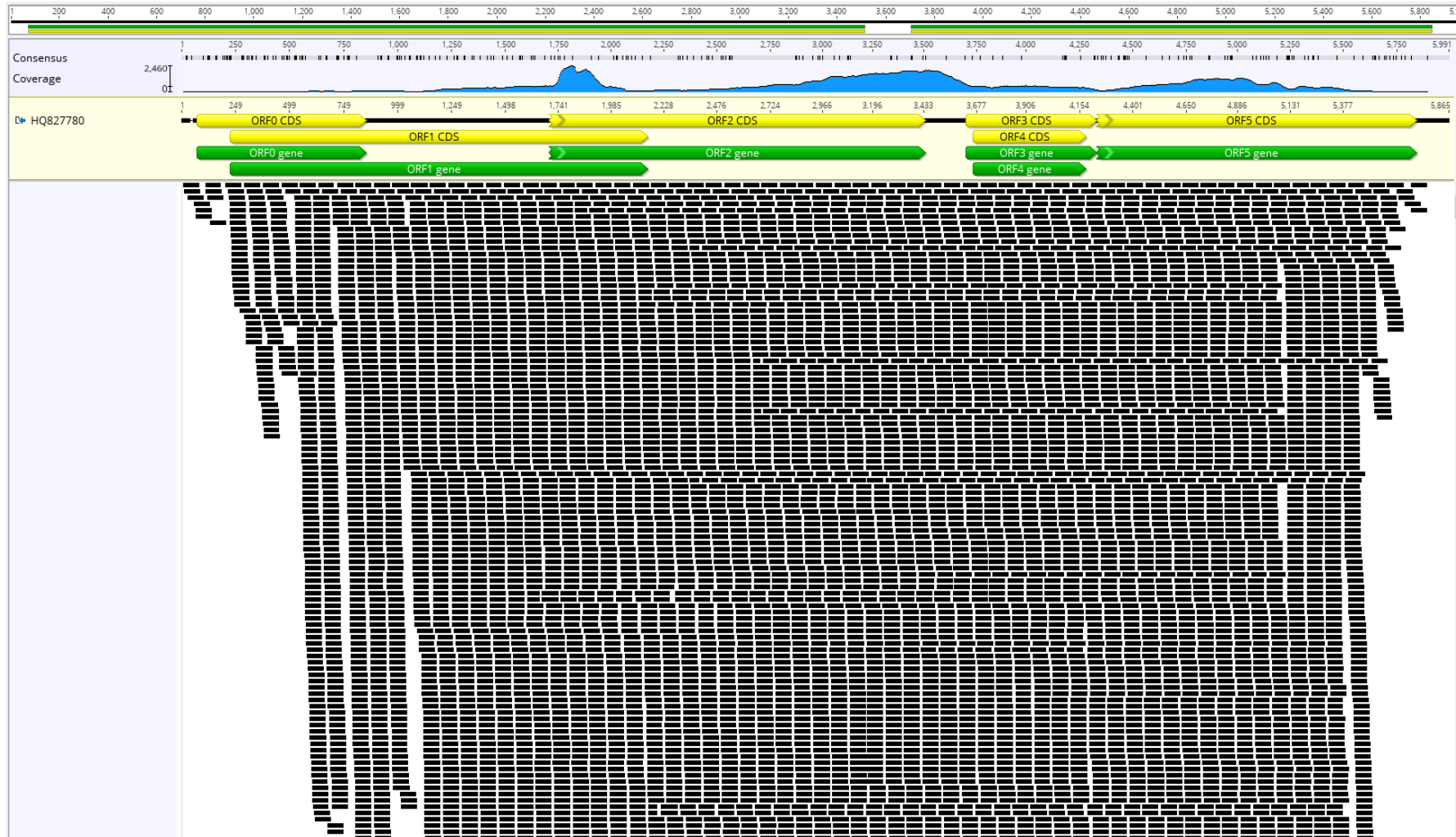
How do we know what virus it is?

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

New species name:
Cotton leaf crinkle dwarf virus
Isolate:
Macon AL_C1

>HQ827780_BR07	100.0							
>GU167940_AR06	98.7	100.0						
>KF906260_BR11	96.4	96.2	100.0					
>KF906261_BR06	96.5	96.4	99.5	100.0				
>KF359946_AR10	96.0	95.8	97.5	97.7	100.0			
>KF359947_AR10	96.0	95.9	97.6	97.7	99.0	100.0		
Macon AL_C1 2018	95.6	95.4	95.1	95.3	95.3	95.2	100.0	

Atypical 96-99%

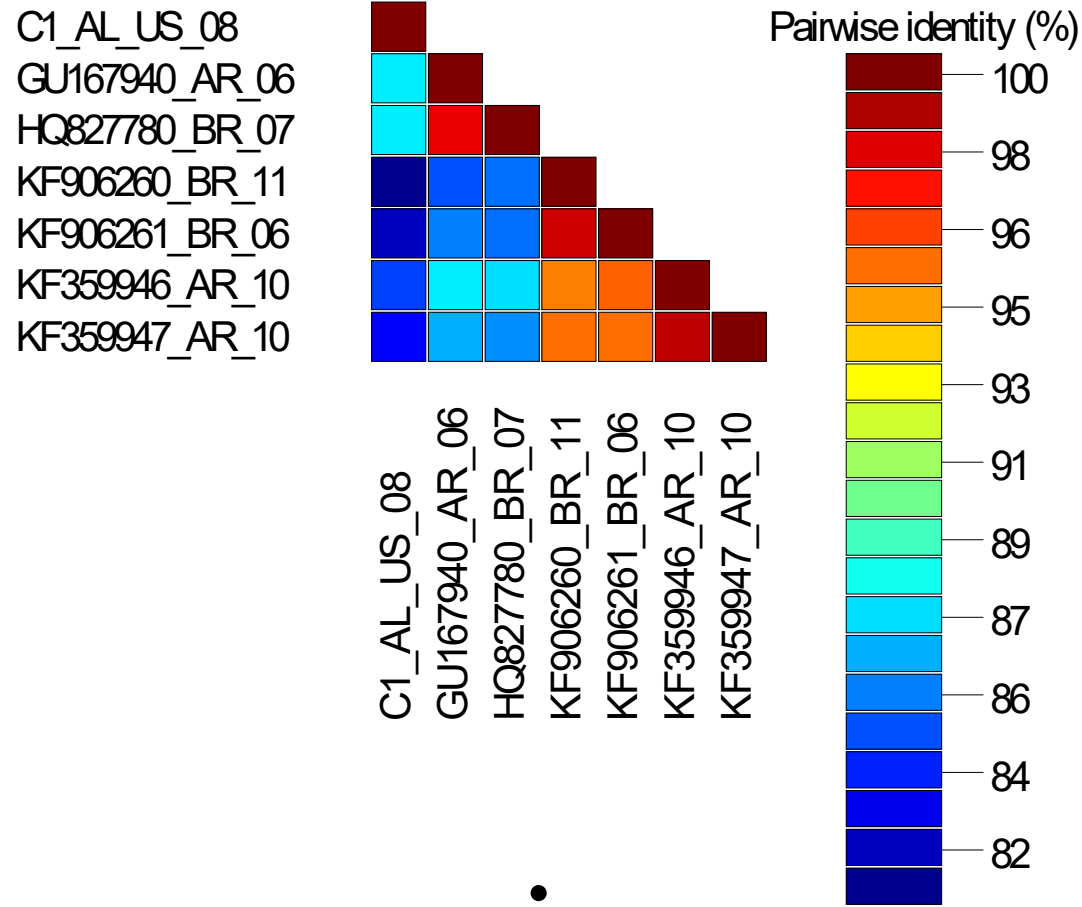
Typical 96-98%

US 2018



ORF 0 amino acid pairwise identity

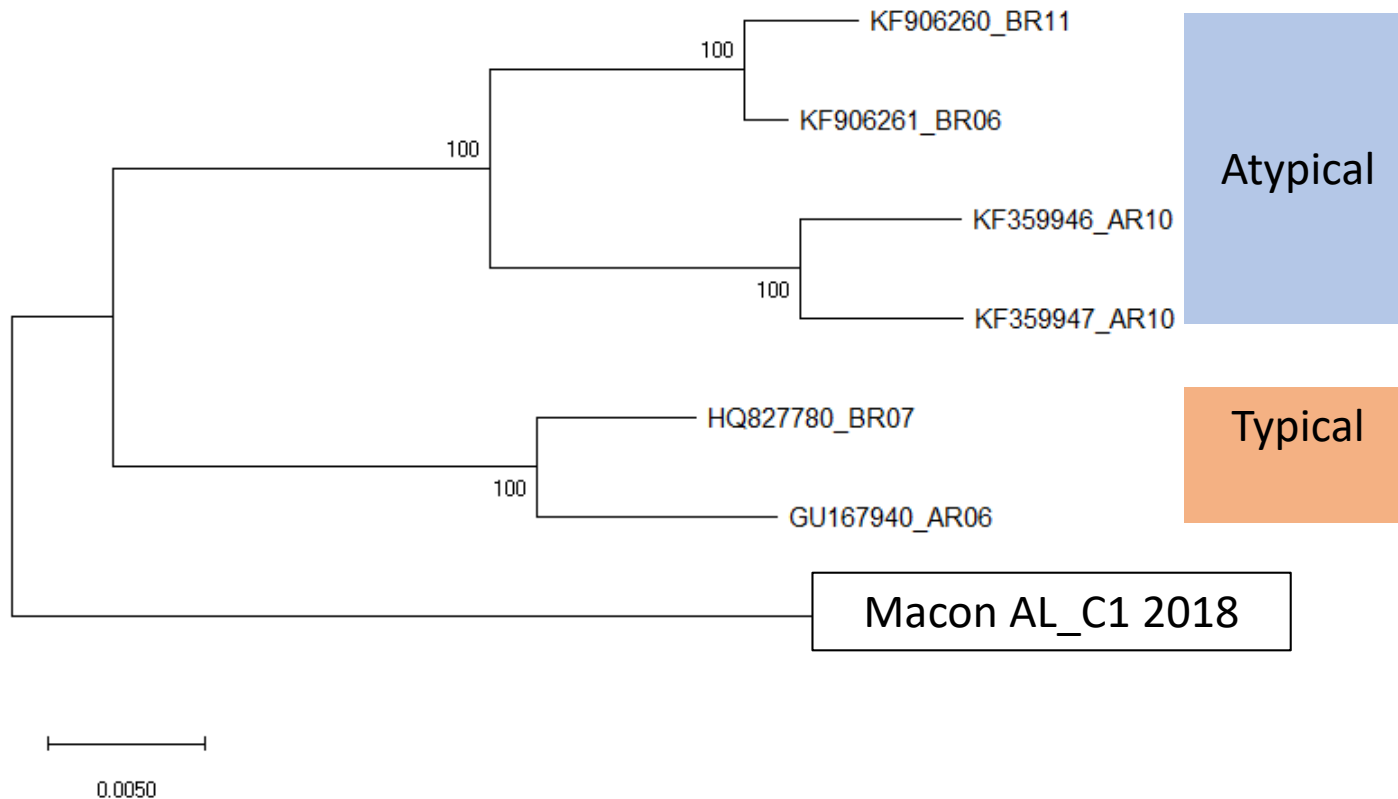
- 82.4-88.5% amino acid sequence pairwise identity
- Other ORFs are ~90-100 %



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
CLRDV3675F	CCACGTAGRCGCAACAGGCGT	CLRDV specific	56 for 10 s and 62 fo 20	310	Sharman et al. 2015
Pol3982R	CGAGGCCTCGGAGATGAACT	CLRDV, CBTv, CABYV, CpCSV, SbDV			
CLRDV_674F	CCGTAGCGGTCATCGTCTTT	CLRDV specific	55	733	Avelar et al. 2018
CLRDV_1407R	TAACCCTGACACAGTGGGGA	CLRDV specific			

– 2019 (*Plant Dis. In press*)

First report of Cotton blue disease in the United States

Sofia Avelar¹, Drew W. Schrimsher², Kathy Lawrence³, and Judith K. Brown¹

¹School of Plant Sciences, University of Arizona, Tucson, AZ

²AGRI AFC

³Dept Entomology and Plant Pathology, Auburn University AL