

Supplementary Figures and Tables

Potential role of viruses in white plague coral disease

Nitzan Soffer, Marilyn E. Brandt, Adrienne M.S. Correa, Tyler B. Smith,
and Rebecca Vega Thurber

Supplemental Table 1. Number of sequence reads before and after quality processing and contamination screening.

Sample	# Raw Reads	# Reads post-QC	# Reads post-QC +Deconseq	# of Similarities to Viral Database	# of similarities to Euk viruses
Healthy Total	68522	21715	11601	4077 (35.14%)	185
Healthy1	46634	11311	1608	429	83
Healthy2	21888	10404	9993	3648	102
Bleached Total	177786	71535	57173	7030 (12.62%)	2478
Blch1	63252	24612	17786	1907	915
Blch2	7035	2423	2336	206	115
Blch3	24432	11337	9409	808	363
Blch4	77208	30529	25037	2743	798
Blch5	5859	2634	2605	1366	287
Bleached and WP Diseased Total	201986	82382	78536	18438 (23.45%)	3601
Blch+Dis1	19087	5892	5677	728	342
Blch+Dis2	7424	3371	3033	459	106
Blch+Dis3	11079	4568	4068	447	304
Blch+Dis4	62793	26118	24953	10317	1197
Blch+Dis5	30660	12147	11924	1904	392
Blch+Dis6	28843	13848	12942	2122	828
Blch+Dis7	42100	16438	15939	2461	432
WP Diseased Total	494119	184309	172690	41382 (23.96%)	12065
Dis1	153916	65278	60629	16488	6760
Dis2	47745	16652	14905	1781	1057
Dis3	70392	26265	25484	8776	1919
Dis4	58522	22674	20186	3562	821
Dis5	83522	28283	27065	4190	934
Dis6	34474	12598	12108	2098	285
Dis7	45548	12559	12313	4487	289
SW Bleached Total	45290	18009	17405	4028 (23.14%)	699
SW Bleached + WP Diseased Total	54645	22689	22685	2965 (13.07%)	515
Grand Total	1044444	400639	360788	77920 (21.60%)	19543

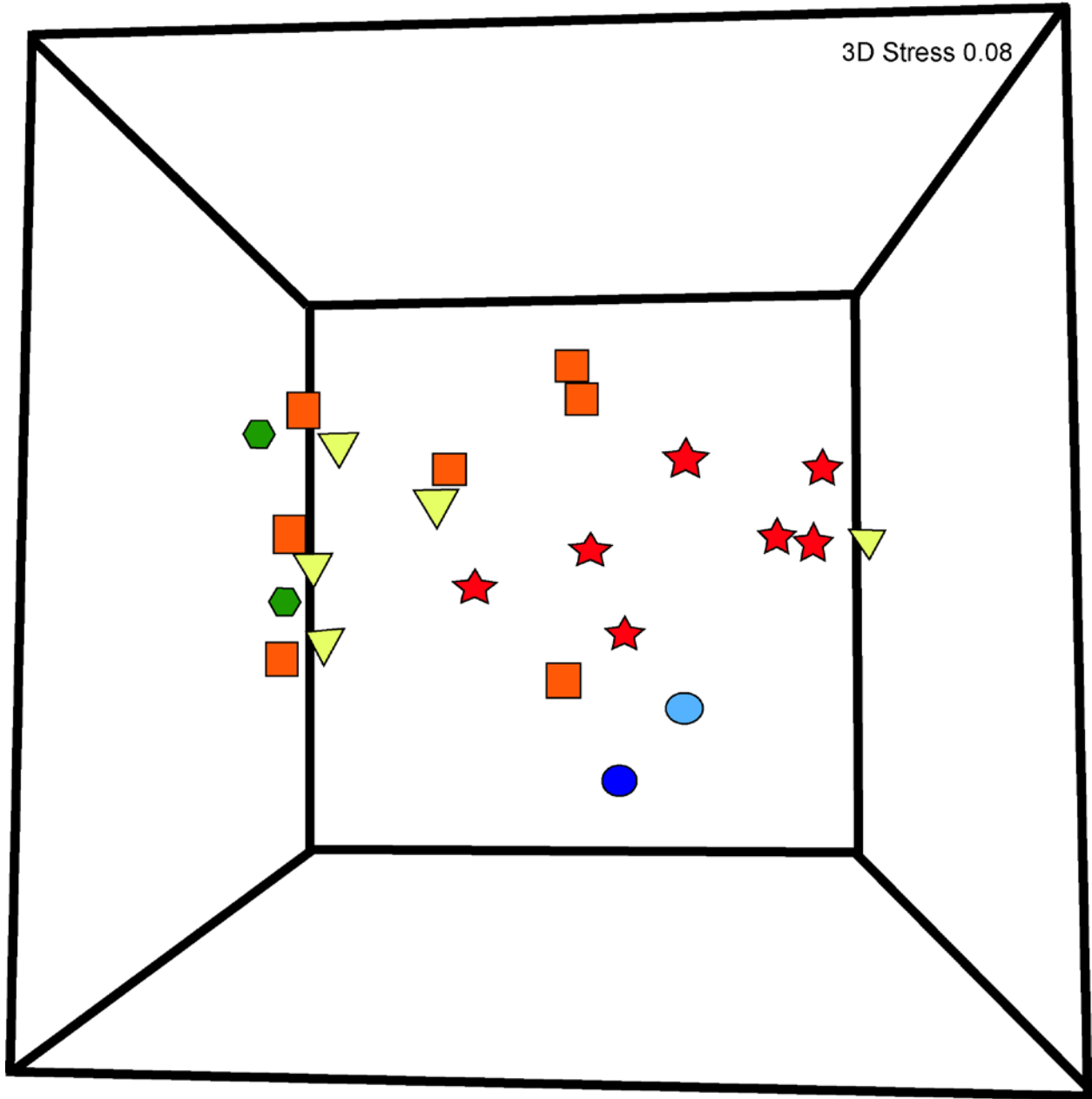
Supplemental Table 2. Similarity Percentage (SIMPER) analysis describing variation among sample types. Mean similarity (Avg. Sim.) among like sample types, and mean dissimilarity (Avg. Diss.) among different sample types are listed. All values are expressed as mean percentages. Viral types contributing to the dissimilarity are denoted by x or y superscript. Y denotes that the sample type from the table vertical column is contributing, while x denotes sample type from the horizontal row axis is contributing. Some samples have less than 1% difference in their contribution, and are labeled with xy.

Sample	Healthy	Bleached	Bleached+ WP Diseased	WP Diseased	SW Bleached	SW Bleched+ WP Diseased
Healthy	Avg. Sim. 70.94 <i>Herpesviridae</i> 81.33 <i>Phycodnaviridae</i> 10.84					
Bleached	Avg. Diss. 46.12 <i>Herpesviridae</i> 27.66 ^x <i>Phycodnaviridae</i> 13.69 ^{xy} <i>Circoviridae</i> 12.55 ^y <i>Mimiviridae</i> 10.88 ^x <i>Nanoviridae</i> 10.43 ^y <i>Ascoviridae</i> 9.63 ^y <i>Poxviridae</i> 9.46 ^y	Avg. Sim. 44.38 <i>Herpesviridae</i> 58.76 <i>Phycodnaviridae</i> 17.97 <i>Mimiviridae</i> 7.64 <i>Ascoviridae</i> 5.44 <i>Poxviridae</i> 5.37				
Bleached + WP Diseased	Avg Diss. 49.72 <i>Herpesviridae</i> 29.26 ^x <i>Mimiviridae</i> 14.68 ^{xy} <i>Phycodnaviridae</i> 13.60 ^{xy} <i>Poxviridae</i> 9.78 ^y <i>Circoviridae</i> 8.86 ^y Satellite 13.12 ^y <i>Papillomaviridae</i> 3.87 ^x	Avg. Diss. 54.23 <i>Herpesviridae</i> 15.55 ^x <i>Phycodnaviridae</i> 13.33 ^{xy} <i>Mimiviridae</i> 12.55 ^y <i>Circoviridae</i> 12.76 ^x <i>Poxviridae</i> 11.89 ^y <i>Nanoviridae</i> 8.94 ^x <i>Ascoviridae</i> 8.34 ^x	Avg. Sim. 42.44 <i>Herpesviridae</i> 57.55 <i>Phycodnaviridae</i> 17.95 <i>Mimiviridae</i> 10.06 <i>Circoviridae</i> 6.87			
WP Diseased	Avg. Diss. 71.99 <i>Herpesviridae</i> 31.75 ^x <i>Circoviridae</i> 21.39 ^y <i>Nanoviridae</i> 12.21 ^y Satellite 9.76 ^y <i>Phycodnaviridae</i> 8.50 ^x <i>Mimiviridae</i> 7.91 ^x	Avg. Diss. 59.84 <i>Circoviridae</i> 19.51 ^y <i>Herpesviridae</i> 19.22 ^x <i>Nanoviridae</i> 15.80 ^y Satellite 11.74 ^y <i>Phycodnaviridae</i> 10.49 ^x <i>Ascoviridae</i> 7.59 ^x <i>Poxviridae</i> 6.48 ^x	Avg. Diss. 61.93 <i>Circoviridae</i> 19.51 ^y Satellite 15.62 ^y <i>Herpesviridae</i> 15.58 ^x <i>Nanoviridae</i> 13.99 ^y <i>Mimiviridae</i> 10.70 ^x <i>Phycodnaviridae</i> 10.29 ^x <i>Poxviridae</i> 7.81 ^x	Avg. Sim. 55.90 <i>Circoviridae</i> 35.94 <i>Herpesviridae</i> 19.51 <i>Nanoviridae</i> 16.62 Satellite 11.09 <i>Phycodnaviridae</i> 9.49		
SW Bleached	Avg. Diss. 75.99 <i>Herpesviridae</i> 40.02 ^x <i>Circoviridae</i> 20.30 ^y Satellite 9.59 ^y <i>Nanoviridae</i> 6.69 ^y <i>Phycodnaviridae</i> 6.62 ^y <i>Mimiviridae</i> 6.55 ^x <i>Polydnaviridae</i> 4.46 ^y	Avg. Diss. 66.47 <i>Herpesviridae</i> 26.56 ^x <i>Circoviridae</i> 17.92 ^y Satellite 10.96 ^y <i>Phycodnaviridae</i> 10.81 ^y <i>Nanoviridae</i> 10.72 ^x <i>Ascoviridae</i> 6.72 ^x <i>Poxviridae</i> 6.41 ^x	Avg. Diss. 66.36 <i>Herpesviridae</i> 23.91 ^x <i>Circoviridae</i> 16.61 ^y Satellite 14.09 ^y <i>Phycodnaviridae</i> 12.02 ^y <i>Mimiviridae</i> 9.93 ^x <i>Nanoviridae</i> 7.40 ^x <i>Poxviridae</i> 7.15 ^x	Avg. Diss. 42.62 <i>Phycodnaviridae</i> 20.28 ^y <i>Herpesviridae</i> 17.73 ^x <i>Circoviridae</i> 15.06 ^x <i>Nanoviridae</i> ^y Satellite 13.84 ^x <i>Polydnaviridae</i> 12.82 ^x <i>Mimiviridae</i> ^y <i>Poxviridae</i> 6.99 ^y 3.06 ^y 2.95 ^x	NA (less than 2 samples)	
SW Bleached + WP Diseased	Avg. Diss. 76.46 <i>Herpesviridae</i> 40.03 ^x <i>Nanoviridae</i> 14.13 ^y <i>Phycodnaviridae</i> 12.03 ^y <i>Circoviridae</i> 59.07 ^y <i>Mimiviridae</i> 6.20 ^x <i>Polydnaviridae</i> 5.06 ^y <i>Poxviridae</i> 3.59 ^y	Avg. Diss. 63.45 <i>Herpesviridae</i> 28.13 ^x <i>Nanoviridae</i> 16.64 ^y <i>Phycodnaviridae</i> 15.59 ^y <i>Circoviridae</i> 10.87 ^y <i>Ascoviridae</i> 7.41 ^x <i>Polydnaviridae</i> 5.98 ^y <i>Poxviridae</i> 5.70 ^x	Avg. Diss. 68.39 <i>Herpesviridae</i> 23.48 ^x <i>Phycodnaviridae</i> 16.01 ^y <i>Nanoviridae</i> 15.54 ^y Satellite 10.62 ^x <i>Mimiviridae</i> 9.68 ^x <i>Poxviridae</i> 7.51 ^x <i>Circoviridae</i> 6.87 ^y <i>Polydnaviridae</i> 4.70 ^y	Avg. Diss. 51.22 <i>Phycodnaviridae</i> 25.17 ^y <i>Circoviridae</i> 19.00 ^x <i>Herpesviridae</i> 15.13 ^x <i>Nanoviridae</i> 11.68 ^y Satellite 10.33 ^x <i>Polydnaviridae</i> 26.67 ^y <i>Poxviridae</i> 2.96 ^y	Avg. Diss. 28.80 <i>Circoviridae</i> 29.48 ^x <i>Nanoviridae</i> 19.87 ^y Satellite 16.91 ^x <i>Phycodnaviridae</i> 14.47 ^y <i>Poxviridae</i> 8.93 ^y <i>Ascoviridae</i> 1.77 ^x	NA (Less than 2 samples)

Supplemental Table 3. Shannon diversity index of mean relative percentage of eukaryotic viral types of all viromes generated. Bold denotes significantly different than Healthy.

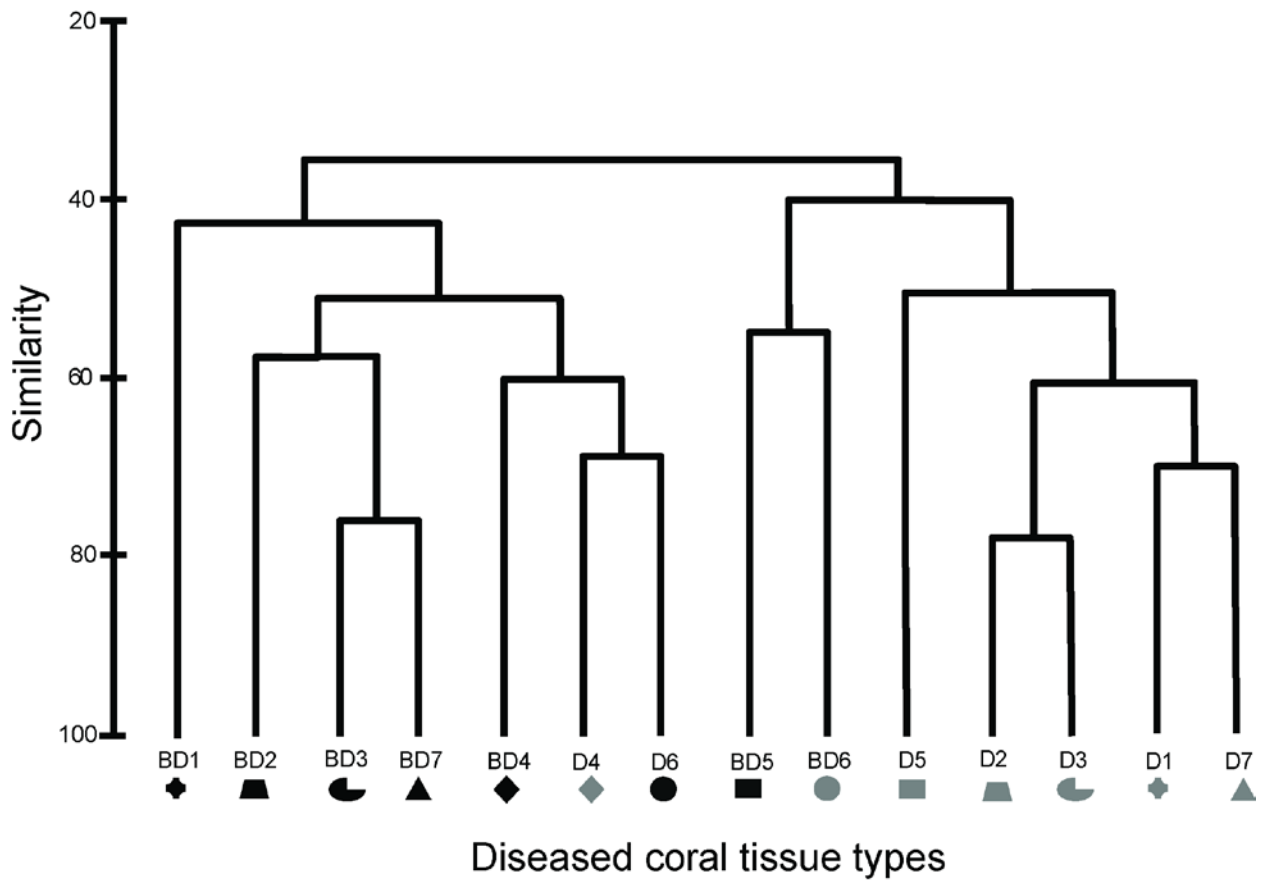
Sample	Shannon Diversity (H')	Standard Error
Healthy	0.98	0.19
Bleached	1.35	0.11
Bleached+WP Diseased	1.30	0.10
WP Diseased	1.62	0.08
SW Bleached	1.80	NA
SW Bleached+WP Diseased	1.87	NA

Supplementary Figure 1. Non-metric multidimensional scaling (MDS) 3D plot comparing viral consortia from viromes. MDS plot was constructed from a Bray-Curtis similarity resemblance matrix based on relative percentage of similarities to viral family.



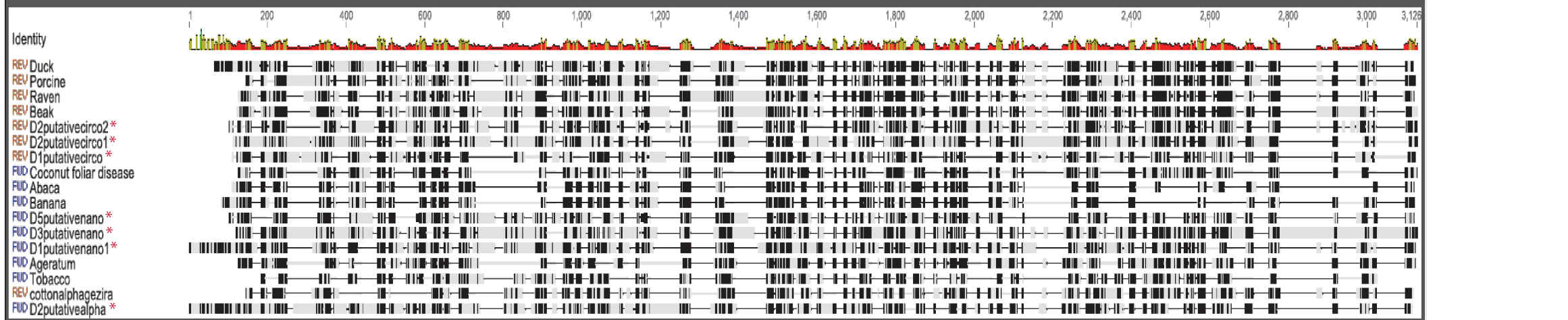
- | | |
|---|---|
|  Healthy |  Diseased |
|  Bleached |  SW Bleached |
|  Bleached+
Diseased |  SW Bleached+
WP Diseased |

Supplementary Figure 2. Cluster analysis of viral consortia from both diseased (intra colony) coral tissue types: Bleached+WP Diseased and WP Diseased. Cluster dendrogram was constructed using group-average linking (similarity was calculated from means of clusters and not the max. or min. values) and Bray Curtis similarities of relative percentages for only Bleached+WP Diseased (BD) and WP Diseased (D) coral colony health states. Matching icons indicate health states from the same coral colony. Black icons are BD tissues, while gray icons are D tissues.



Supplementary Figure 3. Neighbor-Joining phylogenetic tree of SDSDV Rep proteins from WP Diseased health types (D, colored red) and both seawaters (SWD, SWBD, colored blue). Branch length and scores 0-1 represent branch-support based on bootstrap percentages (10,000 bootstraps). Numbers indicate from which sample (i.e. D1.2) the sequence originated. SCSDVs known to infect plants are colored green, while those known to infect animals are colored mustard. Sequence names starting with RW are from reclaimed water (Rosario et al, 2009). A novel SW clade is indicated with a triangle, while stars designate coral specific SCSDV clades.

Supplementary Figure 4. Alignments (above) and ANI distances in percentages (below) of circoviruses, nanoviruses, and satellite DNA genomes to representative SCSDV contigs (noted with red asterisks - i.e: “putative circovirus”) from WP Diseased libraries. As a control, known ANI distances of known circoviruses to each other and known nanoviruses to each other were compared, and statistical differences in ANI were found (d.f. =11, $p < 0.01$). Green bars denote 100% nucleotide agreement, mustard bars denote 30-99% agreement, and red bars denote $< 30\%$ agreement among sequences. Black squares represent $> 25\%$ identity to the consensus, while gray squares represent $< 25\%$ identity to the consensus alignment. Reference whole genomes were from NCBI with respective accession numbers: **circoviruses:** Beak + feather= ref[NC_001944.1; Porcine= ref[NC_001792.2; Raven=ref[NC_008375.1; Duck=ref[NC_007220.1; **nanoviruses:** Abaca bunchy= ref[NC_010319.1]; Banana bunchy=ref[NC_003479.1; coconut folior decay=ref[NC_001465.1; **satellites:** Ageratum alphasatellite=ref[NC_019547.1; Cotton alphasatellite = ref[NC_013593.1; Tobacco necrosis satellite= ref[NC_001557.1]



	Duck	Porcine	Raven	Beak	D2 putative circo2	D2 putative circo1	D1 putative circo	Coconut foliar disease	Abaca	Banana	D5 Putative nano	D3 Putative nano	D1 putativenano1	Ageratum	Tobacco	cottonalpha
Duck																
Porcine	31.7															
Raven	34.8	32.3														
Beak	34.6	32.6	44.4													
D2putativecirco2*	22.8	23.7	22.4	23.7												
D2putativecirco1*	22.3	23.6	23.1	23	21.3											
D1 putativecirco*	21.1	25	20.8	20.4	20.8	19.9										
Coconut foliar disease	20.1	21.1	19.6	19.5	20.6	16.9	19.6									
Abaca	19	20.2	18.6	18.5	18	16.3	21.6	25.6								
Banana	18.6	20	18.2	17.7	17.4	15.6	21.8	25.2	66.9							
D5putativenano*	23.6	21.7	22.2	22.1	21.6	18.6	19	20.8	20.6	20.7						
D3putativenano*	24.5	23.1	24.1	24.9	20	21.6	18.7	18.2	16.9	16.9	19.7					
D1 putativenano1*	24.9	24.5	25.5	24.3	22.3	23.4	21.5	19.9	18.3	19.6	22.4	21.7				
Ageratum	21.3	21.8	21.9	21.2	20.6	20	21.4	29.4	24.7	26.1	20.8	19.2	22.2			
Tobacco	20.3	21.6	21.4	21.3	20	17.3	18.4	22.1	19	19.5	19.6	17.2	21.7	24		
Cottonalpha	22.9	25.2	21.7	23.9	21	19.5	19.7	20.1	19.9	20.5	20.2	19.3	21.9	23	21.8	
D2putativealpha*	26.1	24.6	23	24.1	20.5	23.2	23.6	18	19	18.3	20.9	21.4	22.9	22	19.9	19.3